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A STUDY OF THE PROTEIDS OF THE CORN OR MAIZE KERNEL.

By R. H. CHITTENDEN, Ph. D., *Professor of Physiological Chemistry in Yale University*, and THOMAS B. OSBORNE, Ph. D., *Chemist at the Connecticut Agricultural Experiment Station.*

The character of the proteids present in the kernel or seed of corn or maize, the most important of American cereals, has up to the present time never been investigated to any great extent. In fact, our knowledge of this subject has been practically limited to the observations of Ritthausen[1] concerning "maize fibrin," a body soluble in alcohol, and to the bare statement made by Th. Weyl[2] that the powdered seeds of maize yield to a 10-per cent. solution of sodium chloride a globulin-like substance which, after purification by repeated precipitation with water and re-solution in salt water (10 per cent.), coagulates at 75° C. It is a fair presumption, however, judging from the interesting results obtained by Sidney Martin, Vines and other workers among the vegetable proteids, that the proteids of the corn kernel must doubtless be as numerous and complex as those of other seeds already investigated, and this we have indeed found to be the case. In fact, the subject, while more or less fruitful in results, has proved an exceedingly complex one, on account of the large number of different proteids present in the seed, the small quantities present, and the ease with which several of the bodies, especially the globulins, are apparently converted into other forms. Again, in view of the probable widespread distribution of the vegetable proteolytic ferments, any study of the vegetable proteids normally present in seed or

1 Die Eiweisskörper der Getreidearten, Hülsenfrüchte und Ölsamen, Bonn, 1872, p. 113.
2 Ztschr. für physiolog. Chem. 1, 84.

fruit must necessarily be complicated by possible ferment changes, through which new bodies may be formed.

Our study of the subject has naturally divided itself into three main parts: 1st, a study of the proteids soluble in salt solutions, but insoluble in water; 2d, a study of the proteids soluble both in water and in dilute salt solutions; 3d, a study of the proteid matter soluble in alcohol, but insoluble in water and salt solutions.

I. Proteids Soluble in Salt Solutions, but Insoluble in Water.

Finely ground corn meal[1] extracted with a 10-per cent. solution of sodium chloride yields a slightly opalescent fluid, moderately rich in globulin-like bodies. A simple aqueous extract of ground corn likewise contains more or less globulin held in solution by the salts dissolved from the corn. From both solutions the globulins are slowly precipitated by dialysis of the salts, and partially so by addition of water. They are, moreover, completely precipitated, mixed with other proteids, on saturation of their solutions with ammonium sulphate.

a. *Direct extraction of corn meal with 10-per cent. solution of sodium chloride, and separation of the globulin by dialysis.*

In view of the well known action of water, in the presence of possible ferments, on the globulins of some seeds, the first extractions of the corn kernel were made with salt solutions, with a view to diminishing the possibility of cleavage or dissociation of the normal proteids of the kernel. 5 kilos. of finely ground corn meal were therefore soaked in 8 litres of 10-per cent. solution of sodium chloride, with frequent stirring, for 24 hours, after which the fluid was strained off and the residue placed in a screw-press and squeezed dry. A second extraction of the meal was made with 4 litres of fresh salt solution, and the two extracts united. The solution was then filtered through thick filter-paper, yielding an almost perfectly clear yellowish filtrate.

Tested by fractional heat-coagulation a series of coagulums were obtained commencing at about 35°–40°, a final one making its appearance as the solution was boiled. Further, the clear filtrate from this precipitate gave on addition of acetic acid a

[1] All the corn used in these experiments was of one variety, known as White Dent, and was freshly ground to a fine powder prior to each experiment.

flocculent precipitate of proteid matter, all of which may be taken as evidence of the presence of a variety of proteid bodies, coagulable and non-coagulable by heat.

The entire salt solution, with its content of proteids and other substances, was then dialysed[1] in a stream of running water for a period of six days, at the end of which time chlorides were entirely removed. As the percentage of salt in the solution began to diminish a heavy white precipitate made its appearance, which steadily increased in amount as the removal of the salt was continued, leaving a perfectly clear fluid containing some coagulable proteids. The globulin precipitated in this manner was separated from the fluid by decantation, washed somewhat with distilled water, and then redissolved in a 10-per cent. solution of sodium chloride. This latter solution was somewhat turbid even after repeated filtration. A portion tested by heat-coagulation gave the following results: At 60° the fluid became decidedly turbid, with formation of flocks at 64°. The temperature was then raised to 68° and the mixture filtered. The clear filtrate became turbid at 74°, with formation of a flocculent precipitate at 79°, which was filtered off when the temperature reached 82°. The filtrate from this precipitate became turbid at 83°, with separation of flocks at 87°. The temperature was then raised to 90° and the precipitate filtered off. This filtrate on being heated to boiling gave no precipitate at first, but as the boiling was continued considerable flocculent matter gradually made its appearance. The largest coagulum was obtained at 79°. If we are to trust the results of this fractional heat-coagulation, the natural inference would be that the globulin-like substance separated by dialysis from the sodium-chloride extract of the corn meal is made up of several distinct globulins, or else that the one globulin possibly present is broken apart into several fragments by the action of heat.

The main portion of this sodium-chloride solution of the globulin was dialysed until the salt was entirely removed, a process which took five days and which left the globulin completely precipitated on the sides of the parchment. The peculiar, somewhat granular, appearance of the deposit led us to examine it under the microscope, when it was seen to be composed entirely of spheroids, thus showing a close approach to crystallisation. The precipitate was

[1] During these long periods of dialysis, putrefactive changes were prevented by the occasional addition of a few drops of a 20-per cent. alcoholic solution of thymol.

collected on a filter, washed with water, alcohol and ether, and when air-dried was found to weigh 10.1 grams, thus indicating about 0.2 per cent. of globulin in the air-dry kernel, on the assumption that the extraction and separation were complete. The filtrate from this last separation of the globulin was almost entirely free from proteid matter, giving only a very slight reaction with Millon's reagent, no further separation of any matter by continued dialysis, neither any precipitate on addition of acetic acid or acetic acid and solution of salt, and only the faintest turbidity on boiling.

A portion of the globulin dissolved in 10-per cent. solution of salt yielded by heat-coagulation much the same results as the previous salt solution of the proteid, viz. turbidity at 58°, formation of flocks at 67°. In the filtrate from this coagulum a second turbidity appeared at 77°, followed by flocks at 87°. Further, in this filtrate another coagulum appeared on boiling the solution, so that while the individual temperatures of coagulation were somewhat different from the preceding, they point to essentially the same general conclusion.

A portion of the globulin was dried at 110° C. until of constant weight, and then analysed with the following results:

Analysis[1] *of Corn Globulin, Preparation A.*

I. 0.3933 gram substance gave 0.2325 gram H_2O = 6.57 per cent. H, and 0.7250 gram CO_2 = 50.42 per cent. C.

II. 0.3693 gram substance gave 0.2182 gram H_2O = 6.55 per cent. H, and 0.6845 gram CO_2 = 50.55 per cent. C.

III. 0.3038 gram substance gave 44.1 cc. N at 10° C. and 761.4 mm. pressure = 17.62 per cent. N.

IV. 0.3067 gram substance gave 44.1 cc. N at 8.5° C. and 757.8 mm. pressure = 17.47 per cent. N.

V. 0.5111 gram substance fused with $KOH + KNO_3$ gave 0.0406 gram $BaSO_4$ = 1.08 per cent. S.

VI. 0.6773 gram substance fused with $KOH + KNO_3$ gave 0.0440 gram $BaSO_4$ = 0.89 per cent. S.

VII. 0.5265 gram substance gave 0.0102 gram ash = 1.93 per cent.

[1] Carbon and hydrogen were determined by combustion in a current of oxygen, in an open tube, the vapors passing over a fairly long layer of coarsely granulated oxide of copper, a shorter layer of chromate of lead, and an anterior roll of freshly reduced metallic copper. Nitrogen was determined by combustion with oxide of copper after the Dumas method, while sulphur was estimated by Hammarsten's modification of Liebig's method.

Percentage Composition of the Ash-free Substance.

							Average.
C	51.41	51.54	..	..	..	..	51.48
H	6.69	6.67	..	..	..	..	6.68
N	..	..	17.97	17.82	..	..	17.90
S	..	..	..	..	1.10	0.91	1.01
O	..	..	..	..	..	..	22.93
							100.00

b. *Direct extraction of corn meal with* 10*-per cent. solution of sodium chloride, and separation of the globulin by ammonium sulphate and dialysis.*

25 kilos. of corn meal were extracted with 50 litres of 10-per cent. salt solution, and the residue of meal extracted a second time with 16 litres of salt solution. The two extracts were united, filtered through paper, and the clear fluid saturated with pure ammonium sulphate. This gave rise to a more or less sticky precipitate composed of all the proteids present in the extract. The precipitate so obtained was filtered off, dissolved in water as far as possible, and then treated with 10-per cent. salt solution. The portion remaining insoluble was washed with salt solution as long as anything was removed, and then reserved for further treatment.

The solutions of the ammonium-sulphate precipitate in water (or rather in dilute ammonium sulphate) and in 10-per cent. sodium chloride were united and dialysed for two weeks, at the end of which time a large amount of globulin had separated from the solution. The clear fluid, however, still contained some globulin, and was therefore reserved for further examination. The separated globulin was filtered off and redissolved in about 2 litres of 10-per cent. solution of sodium chloride, leaving almost no insoluble residue, and the solution again dialysed until the globulin had separated. This required seven days. The product, after being washed and air-dried, weighed 42 grams, and was composed wholly of spheroids. The filtrate from this latter deposit of globulin was again returned to the dialyser, but failed to give any further separation of globulin even after ten days' continued dialysis.

It is to be observed here that the separation of this globulin from a pure sodium-chloride solution is fairly rapid and quite

complete, whereas the presence of ammonium sulphate interferes decidedly with its precipitation by dialysis, doubtless on account of the slower rate of diffusion characteristic of this salt.

The globulin prepared in this manner was readily soluble in salt solution, leaving only a slight insoluble residue. Subjected to heat-coagulation, the following results were obtained with a moderately large amount of globulin in a 10-per cent. solution of sodium chloride: The solution became turbid at 62°, with separation of flocks at 76.5°. The mixture was then kept at 78° for half an hour, after which it was filtered, the filtrate yielding a further turbidity at 83°, with separation of flocks at 93°. This latter coagulation was considerably heavier than the one at 76°. On boiling the solution, there was a slight increase in the amount of coagulum, which was still further increased by the addition of a drop or two of very dilute acid. Hence, this product shows approximately the same general range of heat-coagulation points as the preceding preparation, separated by simple dialysis from the original sodium-chloride extract. As the salt solution of the globulin showed a faint alkaline reaction, another test was made with a solution of approximately the same strength as the preceding, but carefully neutralised. This solution grew turbid at 64°, with formation of flocks at 76.5°. The mixture was filtered at 77°, and on being heated further became turbid at 82.5°, with separation of flocks at 91°. The filtrate from this latter precipitate became turbid on boiling, and yielded a large precipitate on addition of acetic acid. It is thus evident that the proteid dissolved in a neutral salt solution cannot be wholly coagulated by heat, even though the heating be continued for some time. In fact, the solution may be evaporated to dryness on a water-bath, the residue taken up again in water, and in the salt solution which results considerable proteid matter will be found dissolved and non-coagulable on further application of heat. In fact, the great bulk of this product appears to be non-coagulable by heat alone. Variations in the proportion of globulin dissolved in the 10-per cent. salt solution modify the temperatures of coagulation only slightly.

A portion of the substance dried at 110° C. until of constant weight was analysed with the following results, which, aside from a slightly higher percentage of carbon, show a close agreement in composition with the corresponding globulin A.

Analysis of Corn Globulin, Preparation B.

I. 0.3500 gram substance gave 0.2180 gram H_2O = 6.82 per cent. H.

II. 0.3251 gram substance gave 0.1992 gram H_2O = 6.81 per cent. H, and 0.6145 gram CO_2 = 51.55 per cent. C.

III. 0.3717 gram substance gave 0.7021 gram CO_2 = 51.52 per cent. C.

IV. 0.3592 gram substance gave 50.5 cc. N at 2.5° C. and 769.1 mm. pressure = 17.73 per cent. N.

V. 0.7523 gram substance fused with KOH + KNO_3 gave 0.0472 gram $BaSO_4$ = 0.86 per cent. S.

VI. 0.7871 gram substance fused with KOH + KNO_3 gave 0.0491 gram $BaSO_4$ = 0.85 per cent. S.

VII. 0.4622 gram substance gave 0.0025 gram ash = 0.54 per cent.

Percentage Composition of Ash-free Substance.

							Average.
C	..	51.83	51.80	..	..	..	51.82
H	6.85	6.84	..	..	..	..	6.85
N	..	..	..	17.82	..	..	17.82
S	..	..	..	..	0.86	0.85	0.86
O	..	..	..	..	..	..	22.65
							100.00

Another sample of globulin was prepared as follows: 2.5 kilos. of ground corn were extracted with about 10 litres of 10-per cent. solution of sodium chloride, and the extract, together with the washings, after filtration, saturated with ammonium sulphate. The precipitate was dissolved as far as possible in water and in 10-per cent. salt solution, and the united filtrates dialysed until free from chlorides. The deposit of globulin, which gradually formed, was filtered off, washed with alcohol and ether, and dried at 110° C. until of constant weight.

On analysis it gave the following results:

Analysis of Corn Globulin, Preparation C.

I. 0.2307 gram substance gave 0.1442 gram H_2O = 6.95 per cent. H, and 0.4330 gram CO_2 = 51.17 per cent. C.

II. 0.3528 gram substance gave 51.5 cc. N at 11.5° C. and 745.6 mm. pressure = 17.26 per cent. N.

III. 0.3000 gram substance gave 0.0028 gram ash = 0.93 per cent.

Percentage Composition of the Ash-free Substance.

			Average.
C	51.64	..	51.64
H	7.01	..	7.01
N	..	17.42	17.42
S, O	..	..	23.93
			100.00

Still another preparation of globulin was separated in the following manner: 2.5 kilos. of corn meal were extracted with 12 litres of 5-per cent. solution of ammonium chloride, the residue of meal re-extracted with more ammonium-chloride solution, and the united filtrates precipitated with ammonium sulphate. This precipitate was dissolved as far as possible in water and 5-per cent. ammonium-chloride solution, leaving only a small insoluble residue, and the united fluids dialysed in running water for seven days. The globulin which separated was then dissolved in 10-per cent. solution of sodium chloride and redialysed until the salt was entirely removed. The precipitated globulin was then washed with water, alcohol and ether, and after being dried at 110° C. was analysed with the following results:

Analysis of Corn Globulin, Preparation D.

I. 0.1289 gram substance gave 0.0781 gram H_2O = 6.74 per cent. H, and 0.2432 gram CO_2 = 51.43 per cent. C.

II. 0.2639 gram substance gave 39.1 cc. N at 11° C. and 746.5 mm. pressure = 17.57 per cent. N.

III. 0.2561 gram substance gave 0.0012 gram ash = 0.47 per cent.

Percentage Composition of the Ash-free Substance.

			Average.
C	51.67	..	51.67
H	6.77	..	6.77
N	..	17.65	17.65
S	..	..	23.91
O	..	..	
			100.00

In these four preparations we have a good illustration of the general character of the globulin obtainable from the corn or maize kernel by direct extraction with salt solution. The product

appears to be the same whether directly separated from its saline solution by dialysis, or first precipitated by ammonium sulphate and then separated by dialysis. The chemical composition of the four preparations is essentially identical, as seen from the accompanying table:

	Globulin A.	Globulin B.	Globulin C.	Globulin D.	Average.
C	51.48	51.82	51.64	51.67	51.65
H	6.68	6.85	7.01	6.77	6.82
N	17.90	17.82	17.42	17.65	17.69
S	1.01	0.86	23.93	23.91	0.93
O	22.93	22.65			22.91
	100.00	100.00	100.00	100.00	100.00

Many things, however, point to the view that the corn globulin separated from the kernel by the above methods is not a single substance, although we must continually keep in mind the possibility of unknown methods of cleavage which may perchance be facilitated by the very processes made use of in testing the globulin. Foremost among these is the peculiar and more or less variable range of coagulation-points obtained whenever a sodium-chloride solution of the globulin is subjected to fractional heat-coagulation. From the data thus obtained we might argue a variable mixture of phyto-myosin, vitellin, and some non-coagulable globulin. As to myosin, however, saturation of a salt solution of the globulin with salt crystals gives only a small precipitate, indicating but little of this substance. The next point suggestive of mixture is the fact that a portion of the globulin readily undergoes change into an insoluble modification by contact with water or strong salt solutions, while another portion is apparently very resistant to such action. Thus, in the precipitation of the globulin by saturation with ammonium sulphate or by dialysis, more or less insoluble matter is formed (insoluble in 10-per cent. salt solution), but this amount grows less and less as the process is repeated, being apparently co-extensive with the amount of this changeable globulin present. The bulk of the globulin, however, retains its solubility in salt solutions by this treatment, under ordinary circumstances.

c. *Fractional separation of the above corn globulin by various methods.*

Treatment of the purified globulin with 10-per cent. salt solution, as stated above, almost invariably shows the presence of at least

a small amount of substance insoluble in salt solution, possibly due to a partial conversion of the globulin into an albuminate by the action of water. It was further found that addition of water to a 10-per cent. salt solution of the globulin was followed by only a partial precipitation of the proteid. Hence an attempt was made to separate the globulin into three fractions for analysis. Accordingly, 5–6 grams of the air-dry globulin, Preparation A, were treated with about 300 cc. of a 10-per cent. solution of sodium chloride, and the insoluble matter collected on a filter and washed thoroughly with 10-per cent. salt solution. This residue was finally washed with water until the salt was entirely removed, then with alcohol and ether. It weighed 0.57 gram (Preparation A^1).

The salt solution and washings containing the bulk of the globulin, amounting to 430 cc., were diluted with distilled water to 4300 cc., by which an abundant precipitate was obtained. This was allowed to settle, then collected on a filter, washed free from chlorides, and lastly with alcohol and ether. It weighed 1.87 grams (Preparation A^2).

The dilute salt solution containing the remainder of the globulin, representing the more soluble portion, was dialysed in running water until the salt was entirely removed, at the end of which time the globulin had separated wholly in the form of *spheroids*. It was collected on a filter, washed with water, alcohol and ether, and weighed 1.6 grams (Preparation A^3).

These three fractions were dried at 110° C. until of constant weight, and analysed with the following results:

Analysis of Preparation A^2.

I. 0.5068 gram substance gave 0.3082 gram H_2O = 6.75 per cent. H, and 0.9540 gram CO_2 = 51.34 per cent. C.

II. 0.3622 gram substance gave 53.5 cc. N at 8° C. and 759.6 mm. pressure = 18.01 per cent. N.

III. 0.2994 gram substance gave 0.0025 gram ash = 0.83 per cent.

Percentage Composition of the Ash-free Substance.

			Average.
C	51.76	..	51.76
H	6.80	..	6.80
N	..	18.16	18.16
S	..	..	} 23.28
O	..	..	
			100.00

Analysis of Preparation A^3.

I. 0.2220 gram substance gave 0.1382 gram H_2O = 6.91 per cent. H, and 0.4132 gram CO_2 = 50.76 per cent. C.

II. 0.3221 gram substance gave 47.5 cc. N at 6° C. and 762.6 mm. pressure = 18.19 per cent. N.

III. 0.3318 gram substance gave 49.3 cc. N at 5.8° C. and 754.2 mm. pressure = 18.14 per cent. N.

IV. 0.2977 gram substance gave 0.0022 gram ash = 0.74 per cent.

Percentage Composition of the Ash-free Substance.

				Average.
C	51.13	..	..	51.13
H	6.96	..	..	6.96
N	..	18.32	18.27	18.30
S O	..	..	..	} 23.61
				100.00

Analysis of Preparation A^1.

I. 0.3030 gram substance gave 36.5 cc. N at 5.8° C. and 758.8 mm. pressure = 14.79 per cent. N.

II. 0.1914 gram substance gave 0.0112 gram ash = 5.85 per cent.

Percentage of nitrogen in the ash-free substance = 15.59.

The following table shows the relationship of the several products:

	The original globulin.	Portion soluble in the dilute NaCl.	Portion insoluble in the dilute NaCl.	Portion insol. in 10-per ct. NaCl.
	A.	A^3.	A^2.	A^1.
C	51.48	51.13	51.76	..
H	6.68	6.96	6.80	..
N	17.90	18.30	18.16	15.59
S O	1.01 22.93	23.61	23.28	..
Ash	1.93	0.74	0.83	5.85

From this it is to be seen that while there is no very radical point of difference in composition between the first three products, there are changes in the relative percentages which are strongly suggestive of admixture. Certainly that portion of the original globulin insoluble in 10-per cent. salt solution has a noticeably low percentage of nitrogen, surely low enough to account for the

slightly higher percentages of nitrogen in the products A^3 and A^2. From the data here offered there is no positive evidence as to whether A^1 is an alteration-product of the original globulin, or whether it simply represents a small impurity. The former view is certainly the more plausible one, however, especially when it is remembered that this globulin had been dissolved in a salt solution and filtered two or three times previously. Again, it is to be noticed in comparing the two soluble products A^2 and A^3, that the body containing the lower percentage of nitrogen contains the higher percentage of carbon; and further, that in both products the percentage of nitrogen is by this treatment raised so as to correspond closely with the nitrogen content of pure phytovitellin.

In view of these results a portion of corn globulin was next separated into a number of fractions by the solvent action of various strengths of salt solution, and the several fractions studied, especially with reference to their composition and heat-coagulation points.

The globulin (Preparation E) was made from 5 kilos. of freshly ground corn by direct extraction with an abundance of 10-per cent. solution of sodium chloride, the proteids precipitated by saturation of the filtered solution with ammonium sulphate, this precipitate dissolved in water and salt solution, and the filtered fluid dialysed in running water for one week until the globulin had separated.

The entire quantity of globulin, about 10 grams, was then treated directly with one litre of 0.25-per cent. solution of sodium chloride at the temperature of the room and kept in agitation for about 3 hours. The residue was then filtered off and extracted again with one litre of salt solution of the same strength, the solution being kept in contact with the globulin for about 18 hours. This was likewise filtered off and the residue again extracted with two litres of 0.25-per cent. salt solution. A portion of the combined extract obtained in this manner, on being heated, grew slightly turbid at 54°; this turbidity was still slight at 63°, but at 71° a flocculent coagulum appeared. No further coagulum appeared until the solution was boiled, and then only a slight one. The filtrate from this precipitate gave a faint reaction with acetic acid and potassium ferrocyanide. These united extracts were then placed in dialysers and dialysed in running water until the

salt was entirely removed, when the deposited globulin was filtered off, and washed with alcohol and ether. It weighed air-dry 0.18 gram (Preparation E^1).

That portion of the globulin insoluble in 0.25-per cent. salt solution was placed in two litres of 0.5-per cent. solution of sodium chloride, and kept in contact with it for 18 hours or longer at 20° C., after which it was filtered off and treated with a fresh portion of salt solution of the same strength. The extract so obtained became turbid at 56°, and gave a flocculent coagulum at 75°. The filtrate from this coagulum became slightly turbid on boiling and gave a slight precipitate with acetic acid and potassium ferrocyanide. On dialysis of the 0.5-per cent. salt solution 0.527 gram of air-dry globulin was obtained (Preparation E^2).

The remainder of the globulin was next treated with 0.75-per cent. salt solution at 20° C. until all soluble matter was removed. A portion of the solution so obtained, on being heated, became turbid at 59°, with separation of flocks at 72.5°. The filtrate from this coagulum became turbid again at 79° and flocked at 85°. On boiling this filtrate a little more precipitate appeared. By dialysis of the united filtrates 2.32 grams of globulin were obtained (Preparation E^3).

The globulin still undissolved was then treated, after the manner described, with 1.0-per cent. salt solution as long as anything dissolved. This extract coagulated as follows; it became faintly turbid at 63°, with separation of flocks at 79.5°. The filtrate gave a second turbidity at 85°–87°, which increased slightly on boiling. By dialysis, 2.9 grams of globulin were obtained (Preparation E^4).

The residue of the original globulin was next treated with two litres of 2.0-per cent. salt solution, in which it nearly all dissolved. The slight residue was rejected. The extract coagulated as follows: turbid at 79°, with formation of flocks at 90°. The filtrate grew turbid again at 94°, with separation of flocks at 99°. On dialysis of the solution 2.2 grams of globulin were deposited (Preparation E^5). This substance, on being tested anew with 1.0-per cent. salt solution, was found practically insoluble.

The several products enumerated were dried at 110° C. until of constant weight, and then analysed with the following results:

Analysis of that Portion of the Globulin Soluble in 0.25*-per cent. Solution of Sodium Chloride* (E^1).

I. 0.1594 gram substance gave 21.6 cc. N at 4.0° C. and 772.3 mm. pressure = 17.04 per cent. N.

Not enough substance for an ash determination.

Analysis of that Portion of the Globulin Soluble in 0.5*-per cent. Solution of Sodium Chloride* (E^2).

I. 0.2470 gram substance gave 34.9 cc. N at 3.0° C. and 763.3 mm. pressure = 17.63 per cent. N.

II. 0.2170 gram substance gave 0.0075 gram ash = 0.69 per cent. ash.

Percentage of nitrogen in the ash-free substance = 17.74.

Analysis of that Portion of the Globulin Soluble in 0.75*-per cent. Solution of Sodium Chloride* (E^3).

I. 0.3303 gram substance gave 0.2018 gram H_2O = 6.79 per cent. H, and 0.6300 gram CO_2 = 52.02 per cent. C.

II. 0.3222 gram substance gave 0.1987 gram H_2O = 6.86 per cent. H, and 0.6125 gram CO_2 = 51.84 per cent. C.

III. 0.4261 gram substance gave 61.5 cc. N at 4.2° C. and 750.2 mm. pressure = 17.62 per cent. N.

IV. 0.3042 gram substance gave 42.9 cc. N at 3.0° C. and 764.1 mm. pressure = 17.61 per cent. N.

V. 0.4353 gram substance gave 0.0023 gram ash = 0.53 per cent.

Percentage Composition of the Ash-free Substance.

					Average.
C	52.25	52.07	..	..	52.16
H	6.82	6.89	..	..	6.86
N	..	..	17.69	17.68	17.69
S	..	..	..	..	23.29
O	..	..	..	..	
					100.00

Analysis of that Portion of the Globulin Soluble in 1.0*-per cent. Solution of Sodium Chloride* (E^4).

I. 0.3057 gram substance gave 0.1900 gram H_2O = 6.90 per cent. H, and 0.5814 gram CO_2 = 51.87 per cent C.

II. 0.5543 gram substance gave 76.0 cc. N at 3.3° C. and 774.2 mm. pressure = 17.33 per cent. N.

III. 0.3593 gram substance gave 50.0 cc. N at 3.0° C. and 754.3 mm. pressure = 17.16 per cent. N.

IV. 0.4353 gram substance gave 0.0023 gram ash = 0.53 per cent.

Percentage Composition of the Ash-free Substance.

				Average.
C	52.14	..	..	52.14
H	6.93	..	..	6.93
N	..	17.43	17.26	17.35
S	..	..	..	} 23.58
O	..	..	..	
				100.00

Analysis of that Portion of the Globulin Soluble in 2.0-per cent. Solution of Sodium Chloride (E^5).

I. 0.3705 gram substance gave 0.2263 gram H_2O = 6.79 per cent. H, and 0.6995 gram CO_2 = 51.50 per cent. C.

II. 0.3632 gram substance gave 0.2209 gram H_2O = 6.76 per cent. H, and 0.6850 gram CO_2 = 51.46 per cent. C.

III. 0.4940 gram substance gave 70.3 cc. N at 3.0° C. and 755.4 mm. pressure = 17.57 per cent. N.

IV. 0.3434 gram substance gave 0.0028 gram ash = 0.81 per cent.

Percentage Composition of the Ash-free Substance.

				Average.
C	51.92	51.88	..	51.90
H	6.84	6.81	..	6.83
N	..	..	17.71	17.71
S	..	..	..	} 23.56
O	..	..	..	
				100.00

The first thing to be noticed from these results is that only a comparatively small amount of the original globulin is soluble in very weak salt solutions. It is not until the solution contains 0.75 per cent. of salt that much globulin is dissolved. It is further noticeable that the weaker salt solutions have throughout a lower coagulation-point than the stronger salt solutions, and, moreover, that no one of the extracts coagulates completely at a given temperature, but shows some evidence, by a turbidity at least, of several different heat-coagulation points. As regards composition, the nitrogen of the several fractions is practically the same, and as this is apt to be the most variable element in proteid bodies, it is probable that the several fractions have essentially the same

composition. It is further noticeable that the composition of the fractions E^3, E^4 and E^5 is practically identical with the composition of the globulins A and B, although the average content of carbon in the former products is a trifle higher. Of the 10 grams of air-dry globulin started with, in this experiment, 8.12 grams were recovered in the several fractions. There was obviously some loss, especially in the many filtrations, so that this deficiency is not to be considered as representing the amount of globulin or alteration product insoluble in the 2.0-per cent. salt solution. The actual amount of insoluble matter was certainly far smaller than this.

It would have been interesting to have determined the percentage of nitrogen in the insoluble portion, but the quantity was so small and it was so mixed with shreds of filter-paper that the result in this case could have had little value.

The relationship of these several fractions or portions of the original globulin is clearly shown in the following table:

Portion soluble in NaCl	0.25 per ct.	0.50 per ct.	0.75 per ct.	1.0 per ct.	2.0 per ct.
C	..	..	52.16	52.14	51.90
H	..	..	6.86	6.93	6.83
N	17.04	17.74	17.69	17.35	17.71
S } O }	..	..	23.29	23.58	23.56
Coagulation-points,[1] 1st.	54°, 71°	56°, 75°	59°, 72.5°	63°, 79.5°	79°, 90°
" " 2d.	..	..	79°, 85°	85°, 87°	94°, 99°

This experiment, then, so far as it goes, corroborates the idea that the original globulin is a mixture of closely allied bodies possessed of different heat-coagulation points and of different degrees of solubility in dilute salt solutions. This method of treatment, however, is insufficient to bring about a complete separation of the individual bodies, the separate fractions obtained above being evidently themselves more or less mixtures, as indicated by their heat-coagulation points and their resemblance in chemical composition. This might indeed be possible, even if there were only two globulins present, for, as is well known, it is often as difficult to bring about a sharp separation of certain proteid bodies, having many points in common, as it is to separate a mixture of several fats.

[1] The first figure of each pair represents the appearance of a turbidity, the second figure the formation of flocks.

Another and more successful separation of the globulin into its possible constituents was made by means of fractional heat-coagulation. Obviously, the products so obtained, or at least a portion of them, are coagulated by the process; but if the substance is composed simply of a single body, the several products or coagulums might naturally be expected to have the same composition.

In this experiment, 10 grams of globulin B were dissolved in 10-per cent. salt solution, filtered, the clear fluid heated to 50° in a roomy water-bath and a slight excess of very dilute hydrochloric acid added, by which a copious precipitate was obtained, doubtless an acid compound, leaving the fluid perfectly neutral. This precipitate was washed with salt solution, water, alcohol and ether, and weighed, air-dry, 1 gram (Preparation B^1).

The perfectly neutral filtrate, on being heated further, became turbid at 57°, with formation of flocks at 66°. The temperature, however, was raised to 70°, where it was held some time, and the precipitate finally filtered off and washed with salt solution, water, alcohol and ether. It weighed, air-dry, about 1 gram (Preparation B^2). The filtrate became turbid again at 72°, and at 75° a flocculent precipitate made its appearance, which was finally filtered off when the temperature reached 81°. This was treated like the preceding products, and when air-dry weighed 1 gram (Preparation B^3).

The clear filtrate from this latter coagulum was dialysed free from chlorides, when there resulted a separation of a globulin-like substance, which, after washing with water, alcohol and ether, weighed, air-dry, 1.43 grams (Preparation B^4).

These several products, on being dried at 110° C. until of constant weight, gave on analysis the following results:

Analysis of the HCl Precipitate at 50° (B^1).

I. 0.3539 gram substance gave 48.7 cc. N at 2.5° C. and 767.8 mm. pressure = 17.33 per cent. N.

II. 0.4731 gram substance gave 0.0016 gram ash = 0.34 per cent.
Percentage of nitrogen in ash-free substance = 17.39.

Analysis of the Coagulum at 66°–70° (B^2).

I. 0.3687 gram substance gave 47.3 cc. N at 2.5° C. and 767.8 mm. pressure = 16.15 per cent. N.

II. 0.3855 gram substance gave 0.0017 gram ash = 0.44 per cent.
Percentage of nitrogen in the ash-free substance = 16.21.

Analysis of the Coagulum at 75°–81° (B^3).

I. 0.3361 gram substance gave 44.9 cc. N at 2.5° C. and 766.1 mm. pressure = 16.78 per cent. N.

II. 0.2015 gram substance gave 0.0004 gram ash = 0.19 per cent.
Percentage of nitrogen in the ash-free substance = 16.81.

Analysis of the Globulin separated by Dialysis (B^4).

I. 0.2993 gram substance gave 0.1810 gram H_2O = 6.72 per cent. H, and 0.5674 gram CO_2 = 51.72 per cent. C.

II. 0.2595 gram substance gave 0.1596 gram H_2O = 6.83 per cent. H, and 0.4910 gram CO_2 = 51.60 per cent. C.

III. 0.3300 gram substance gave 47.9 cc. N at 2.5° C. and 762.2 mm. pressure = 18.14 per cent. N.

IV. 0.3265 gram substance gave 0.0016 gram ash = 0.49 per cent.

Percentage Composition of the Ash-free Substance.

				Average.
C	51.97	51.85	..	51.91
H	6.75	6.86	..	6.81
N	..	..	18.22	18.22
S O	.. ..	.. ..	.. ..	23.06
				100.00

A comparison of these results shows plainly that the globulin separable from corn meal by the process previously described, as in Preparation B, is composed of at least two dissimilar proteids, or, less probably, is broken into such bodies by the process of heat-coagulation. The products coagulable below 80° are characterised by a comparatively low percentage of nitrogen, while the product (B^4) separated by dialysis of the filtrate from the coagulum at 80° has a higher content of nitrogen than the original globulin, resembling in this respect Preparation A^2, the more insoluble portion of globulin A^1. This relationship in chemical composition is clearly seen from the following table:

	Globulin B.	Prep. B^1.*	Prep. B^2.	Prep. B^3.	Prep. B^4.	Prep. A^2.
C	51.82	..	..	..	51.94	51.76
H	6.85	..	..	..	6.81	6.80
N	17.82	17.39	16.21	16.81	18.22	18.16
S, O	23.51	..	..	..	23.06	23.28

*In considering these results it must be remembered that Preparation B^1 is probably nothing more than an acid compound of the mixed globulins.

This close relationship in composition between the products A^2 and B^4 is strongly suggestive of their being the same body, viz., a form of phyto-vitellin especially characterised by non-coagulation below 80° and by very incomplete coagulation above that temperature, even when its solution in sodium chloride is heated to 100°.

Another point to be noted in the above experiment is, that of the 10 grams of globulin (B) started with, only 4.43 grams were recovered in the form of the products B^1 — B^4, just described. Naturally some loss must have occurred, especially through possible incomplete separation during dialysis of the final solution, but evaporation of the salt-free fluid from which globulin B^4 had separated revealed the presence of about 1.5 grams of non-coagulable matter, either derived from, or present in, the original globulin. The substance so obtained gave a red color with cupric sulphate and potassium hydroxide, the usual proteid reactions with Millon's reagent, no reaction with acetic acid, but a heavy precipitate with acetic acid and potassium ferrocyanide. Addition of acetic acid to a 10-per cent. salt solution of the substance gave a heavy precipitate, possibly in part of globulin, which was still further increased by saturation of the acid fluid with salt. Saturation of a neutral solution of the substance with salt gave no precipitate whatever. Nitric acid produced a distinct turbidity, and addition of an equal volume of alcohol yielded a heavy precipitate.

From this it is plainly evident that in the separation or breaking apart of the original globulin by heat-coagulation there is formed in small quantity a body, or bodies, non-coagulable by heat and readily soluble in water; in other words, bodies resembling proteoses. That such soluble substances were not present in the original globulin was proved by boiling a small portion of globulin B in water and then filtering the hot solution. The filtrate

gave no reaction whatever for proteids with the biuret test. In other words, the original globulin contained no proteose-like substances, but when dissolved in salt solution and subjected to heat-coagulation such bodies were apparently formed. This was demonstrated in several ways, among others as follows: A portion of globulin B was suspended in water, and the emulsion quickly poured into a 10-per cent. salt solution heated to 75° C. A nearly complete solution resulted, the temperature not falling below 65°. The temperature was immediately raised to 100° and the boiling continued for some minutes, thus killing any possible ferment present, after which the coagulum was filtered off. The somewhat turbid filtrate was evaporated to dryness on a water-bath, a little coagulum forming during the process, and the residue taken up in water. Addition of acetic acid to the strong salt solution produced a heavy precipitate of the vitellin-like proteid, but there still remained in solution some non-precipitable proteid, giving a distinct pink color with the biuret test. While, then, this so-called corn globulin can be approximately separated by heat-coagulation into two dissimilar globulins, there is apparently formed at the same time, presumably by hydrolysis of the less resistant globulin, a small amount of proteose-like bodies, the quantity of which appears to be dependent in part upon the duration of the heating.

The vitellin with 18 or more per cent. of nitrogen is especially characterised, as already indicated, by its pronounced insolubility in weak salt solutions, especially when cold. It is likewise more prone to separate in the spheroidal state. By making use of these facts we were able to separate it directly from the mixed globulin without recourse to coagulation. The method was as follows: 8 grams of globulin B were dissolved in 300 cc. of 5-per cent. salt solution, and the fluid filtered. The clear solution was then diluted with distilled water to about 2 litres, whereby a heavy precipitate resulted. On warming to about 45° C., however, a nearly clear solution was obtained, which on gradual cooling to 8° C. deposited at the end of 48 hours a precipitate of spheroids. This precipitate was then filtered off, dissolved in as little salt solution as possible (warmed to 50° C.), and diluted with distilled water at the same temperature, until a permanent turbidity began to make its appearance. The globulin which separated from this solution on cooling, was wholly soluble in the salt fluid when warmed to 50° C. As the amount of globulin which separated by this treatment was

small, the entire mixture was further diluted with an equal volume of water and the resulting precipitate collected. It was then partly dissolved in about 2 litres of quite dilute salt solution, the mixture heated to 50°, and 20-per cent. salt solution added until the globulin had entirely dissolved. On cooling this solution very slowly, ultimately near to 0°, a considerable deposit of very large spheroids resulted, which, after being washed with water, alcohol and ether, weighed, air-dry, 1.5 grams. This product (B^5), on being dried at 110° C. until of constant weight, gave by analysis the following results:

Analysis of the Globulin B^5.

I. 0.4311 gram substance gave 0.2658 gram H_2O = 6.85 per cent. H, and 0.8227 gram CO_2 = 52.05 per cent. C.

II. 0.3338 gram substance gave 49 cc. N at 3° C. and 754.2 mm. pressure = 18.10 per cent N.

III. 0.4458 gram substance gave 0.0019 gram ash = 0.42 per cent.

Percentage Composition of the Ash-free Substance.

			B^5.	B^4.	A^2.	Globulin B.
C	52.26	..	52.26	51.94	51.76	51.82
H	6.88	..	6.88	6.81	6.80	6.85
N	..	18.17	18.17	18.22	18.16	17.82
S	..	..	22.69	23.06	23.28	0.86
O	..	..				22.65
			100.00	100.00	100.00	100.00

Comparison of the analysis of this product with that of the original mixed globulin B shows that the nitrogen has been raised to correspond with the percentage in the preceding preparations of vitellin, notably B^4 and A^2, while the carbon is a trifle higher than in the latter products. Evidently, then, this simple process is sufficient to separate the vitellin from the mixed globulin; the more soluble globulin, with its lower content of nitrogen, remaining in the several solutions.

It is thus evident that the corn or maize globulin originally referred to by Weyl is a mixed substance, composed of at least two dissimilar globulins, one of which approximates in composition to phyto-myosin, the other to phyto-vitellin, although both bodies possess peculiarities of reaction not exactly in accord with the reactions of these two substances as usually described.

d. *Extraction of corn meal with water, and separation of the globulin by ammonium sulphate and dialysis.*

When corn meal is extracted with water there results a solution rich in salts, especially phosphates of the alkalies, in which more or less globulin is dissolved. Direct dialysis of such a solution is followed by separation of the dissolved globulin. It can likewise be precipitated, together with the other proteids present, by saturation of the solution with ammonium sulphate. A concentrated solution, obtained by extracting corn meal with water, when heated grows turbid at about 50° C. (the exact point depending on the concentration of the fluid), with formation of a flocculent coagulum at about 60°. At 64° the solution again becomes turbid, with formation of a second flocculent coagulum at 75°.

The globulin was separated as follows: 6.5 kilos. of corn meal were treated with 10 litres of water, with more or less stirring, for some hours, and the residue of meal re-extracted in the same manner. The united solutions, filtered clear, were saturated with ammonium sulphate. The precipitate so obtained was dissolved as far as possible in water and in 10-per cent. salt solution, leaving quite an insoluble residue. The united aqueous and salt solutions, after filtration, were dialysed until the greater portion of the salt was removed, when there resulted a decided separation of globulin. This globulin was far more insoluble in 10-per cent. solution of sodium chloride than any previous product. In other words, a much larger proportion of this originally soluble globulin passed into an insoluble modification during the process of dialysis, etc., than any product previously separated. That portion still soluble in 10-per cent. salt solution coagulated as follows: turbid at 54°–60°, with formation of flocks at 67°–71°. A second turbidity appeared in the filtered fluid at 69°–72°, but this was very slight, and was not much increased even when the solution was boiled. Addition of acetic acid, however, gave a distinct precipitate. On dialysis of the sodium-chloride solution, the dissolved globulin was deposited. It amounted to only 0.3 gram when air-dry (Preparation F). Dried at 110° C., it yielded by analysis 16.80 per cent. of nitrogen, thus agreeing approximately both in content of nitrogen and in coagulation-point with that portion of the mixed globulin B coagulable below 80°, viz., B^2 and B^3.

0.2593 gram substance gave 35.2 cc. N. at 2.5° C. and 754.9 mm. pressure = 16.80 per cent. N.

There was not enough substance for an ash determination.

A larger quantity of this globulin, sufficient for a more complete analysis, was obtained from another portion of the corn meal as follows: 5 kilos. of freshly ground corn were thoroughly extracted with water, and the filtered solution precipitated by the addition of ammonium sulphate to saturation. This precipitate was treated with water, and the globulin soluble in the dilute ammonium sulphate solution formed was, after filtration, separated from the fluid by dialysis. After washing the separated globulin with water, alcohol and ether, it weighed, air-dry, 2 grams (Preparation G[1]).

Dried at 110° C. it gave on analysis the following results:

Analysis of Corn Globulin, Preparation G[1].

I. 0.1973 gram substance gave 0.1243 gram H_2O = 6.99 per cent. H, and 0.3784 gram CO_2 = 52.30 per cent. C.

II. 0.3343 gram substance gave 0.2071 gram H_2O = 6.88 per cent. H, and 0.6392 gram CO_2 = 52.14 per cent. C.

III. 0.3594 gram substance gave 50.5 cc. N at 17.1° C. and 758.5 mm. pressure = 16.57 per cent. N.

IV. 0.4590 gram substance fused with $KOH + KNO_3$ gave 0.0433 gram $BaSO_4$ = 1.28 per cent. S.

V. 0.3159 gram substance gave 0.0025 gram ash = 0.79 per cent.

Percentage Composition of the Ash-free Substance.

					Average.
C	52.71	52.56	..	..	52.64
H	7.04	6.94	..	..	6.99
N	..	..	16.70	..	16.70
S	..	..	..	1.28	1.28
O	..	..	..	..	22.39
					100.00

A duplicate of this last product was prepared from another portion of corn meal (5 kilos.) in essentially the same manner. The meal was thoroughly extracted with water, the filtered extract precipitated by saturation with ammonium sulphate, the precipitated proteids filtered off and soaked in water, by which the globulin and some other substances were dissolved. From this dilute ammonium sulphate solution the globulin was separated by dialysis of the salt, and after being washed with water, alcohol and ether, weighed, when air-dry, 2 grams (Preparation H[1]).

Dried at 110° C. and analysed, it yielded the following results:

Analysis of Corn Globulin, Preparation H[1].

I. 0.2825 gram substance gave 0.1769 gram H_2O = 6.96 per cent. H, and 0.5425 gram CO_2 = 52.37 per cent. C.

II. 0.1553 gram substance gave 0.0989 gram H_2O = 7.07 per cent. H, and 0.2987 gram CO_2 = 52.45 per cent. C.

III. 0.4200 gram substance gave 59.0 cc. N at 17.2° C. and 765.1 mm. pressure = 16.71 per cent. N.

IV. 0.3118 gram substance gave 0.0021 gram ash = 0.67 per cent. (ash mainly oxide of iron).

V. 0.5500 gram substance fused with $KOH + KNO_3$ gave 0.0530 gram $BaSO_4$ = 1.32 per cent. S.

Percentage Composition of the Ash-free Substance.

					Average.
C	52.72	52.80	..	..	52.76
H	7.00	7.11	..	..	7.05
N	..	..	16.82	..	16.82
S	..	..	..	1.32	1.32
O	..	..	..	..	22.05
					100.00

From this description it is obvious that only a portion of the globulins contained in the corn-kernel can be withdrawn by water; in other words, the dilute salt solution which results when corn meal is extracted with water is capable of dissolving only a portion of the globulins present. More important, however, is the fact that the globulin which is so dissolved represents simply that portion of the mixed globulins, previously described, coagulating under 80°, or, to be more exact, it is apparently a body coagulating completely at 75° C. and with a content of nitrogen approximating 16.8 per cent. Furthermore, it is a globulin excessively prone to become insoluble in salt solution by long contact with water or with strong solutions of salt. In other words, it agrees closely with the general descriptions of vegetable myosin except in its peculiar coagulation-points. This view is still further substantiated by the almost complete agreement in composition with animal myosin. This relationship in chemical composition can be clearly seen from the following table:

	Corn myosin. F[1].	Corn myosin. G[1].	Corn myosin. H[1].	Coagulum at 75° from mixed globulin. B[3].	Animal myosin.*
C	..	52.64	52.72	..	52.82
H	..	6.99	7.05	..	7.11
N	16.80	16.70	16.82	16.81	16.77
S	..	1.28	1.32	..	1.27
O	..	22.39	22.05	..	21.90

* Average of the analyses of 13 different preparations of animal myosin from various sources. See Studies from Laboratory of Physiological Chemistry, Yale University, 3, 133.

Hence, by this method of extracting corn meal directly with water, and by the use of the method of separation already described, only one globulin is obtained, with a coagulation-point of about 70° and agreeing substantially in other respects with the general properties of vegetable myosin. Doubtless the trace of a coagulum obtained at higher temperatures and on the addition of acid is due to a slight admixture of the vitellin-like body. The above statement being correct, it is obvious that the residue of corn meal remaining after complete extraction with water should yield to 10-per cent. salt solution a globulin with 18 per cent. or more of nitrogen unmixed with myosin, providing the latter body has been completely withdrawn from the tissue of the seed.

e. *Extraction of corn meal with 10-per cent. solution of sodium chloride, after previous extraction with water, and separation of the globulin by direct dialysis, and by precipitation with ammonium sulphate and dialysis.*

Extraction of corn meal with 10-per cent. salt solution, after previous extraction with water, yields a solution containing considerable globulin, which can be separated in part by addition of water and completely, or nearly so, on dialysis.

6.5 kilos. of corn meal, after thorough extraction with water (Extract F), were treated several times with an abundance of 10-per cent. salt solution, and the dissolved proteids precipitated from the filtered extract by saturation with pure ammonium sulphate. The precipitate was dissolved, so far as possible, in water and 10-per cent. salt solution, and the united fluids dialysed in running water until the bulk of the salt was removed. The globulin separated in this manner dissolved only partially in 10-per cent. salt solution. The soluble matter coagulated as follows: *slight turbidity at* 60°–63°, *with separation of a few flocks at* 71°–72°. The filtrate from this coagulum grew turbid again at 81°–82°, flocking at 91°–95°. Addition of acetic acid to this filtrate gave quite an abundant precipitate. This globulin dissolved by the 10-per cent. salt solution was again separated from the fluid by dialysis. The precipitate so obtained, when washed with water, alcohol and ether, weighed, air-dry, 4.12 grams (Preparation F^2). It was almost entirely soluble in 10-per cent. salt solution, and when subjected to heat-coagulation yielded only a slight turbidity at 63°, with separation of a few flocks at 73°. On heating this filtrate nothing further appeared other than a trifling

turbidity, even when the solution was boiled. Addition of acetic acid, however, gave a heavy precipitate. Evidently, therefore, this body is characterised especially by non-coagulation by heat, in a neutral solution, the trifling coagulum observed being unquestionably due to a trace of myosin.

Dried at 110° C. until of constant weight, and analysed, the following results were obtained:

Analysis of Corn Globulin, Preparation F².

I. 0.3781 gram substance gave 0.2295 gram H_2O = 6.74 per cent. H, and 0.7205 gram CO_2 = 51.96 per cent. C.

II. 0.3293 gram substance gave 0.2031 gram H_2O = 6.85 per cent. H, and 0.6253 gram CO_2 = 51.79 per cent. C.

III. 0.3401 gram substance gave 48.8 cc. N at 3° C. and 766.9 mm. pressure = 17.99 per cent. N.

IV. 0.3972 gram substance gave 57 cc. N at 3° C. and 756.6 mm. pressure = 17.96 per cent. N.

V. 0.7561 gram substance fused with $KOH + KNO_3$ gave 0.0337 gram $BaSO_4$ = 0.61 per cent. S.

VI. 0.8611 gram substance fused with $KOH + KNO_3$ gave 0.0446 gram $BaSO_4$ = 0.71 per cent. S.

VII. 0.3951 gram substance gave 0.0009 gram ash = 0.22 per cent.

Percentage Composition of the Ash-free Substance.

							Average.
C	52.07	51.90	..	..	..	..	51.99
H	6.75	6.86	..	..	..	..	6.81
N	..	..	18.03	18.00	..	..	18.02
S	..	..	..	..	0.61	0.71	0.66
O	..	..	..	..	..	..	22.52
							100.00

The character of this product was verified by another preparation as follows:

5 kilos. of finely ground corn, after being thoroughly extracted with water (Preparation G), were treated with an abundance of 10-per cent. salt solution, and the filtered extract directly dialysed until the salt was entirely removed. The precipitate which separated was washed with water, then redissolved in 10-per cent. salt solution, filtered from the insoluble residue, and again dialysed until the globulin had completely separated once more. This final product was very soluble in salt solutions, and weighed, after being washed with alcohol and ether, 5 grams (Preparation G²).

A portion dried at 110° C. gave on analysis the following results:

Analysis of Corn Globulin, Preparation G².

I. 0.3063 gram substance gave 0.1857 gram H_2O = 6.73 per cent. H, and 0.5607 gram CO_2 = 49.91 per cent. C.

II. 0.3205 gram substance gave 0.1925 gram H_2O = 6.76 per cent. H, and 0.5882 gram CO_2 = 50.04 per cent. C.

III. 0.4245 gram substance gave 63.3 cc. N at 17.0° C. and 764.5 mm. pressure = 17.73 per cent. N.

IV. 0.5045 gram substance fused with $KOH + KNO_3$ gave 0.0355 gram $BaSO_4$ = 0.96 per cent. S.

V. 0.4490 gram substance fused with $KOH + KNO_3$ gave 0.0315 gram $BaSO_4$ = 0.96 per cent. S.

VI. 0.5435 gram substance gave 0.0125 gram ash = 2.30 per cent.

VII. 0.2865 gram substance gave 0.0062 gram ash = 2.16 per cent.

Percentage Composition of the Ash-free Substance.

						Average.
C	51.04	51.17	..	..	..	51.10
H	6.88	6.91	..	..	..	6.90
N	..	..	18.13	..	..	18.13
S	..	..	..	0.98	0.98	0.98
O	..	..	..	..	..	22.89
						100.00

A third preparation of the same order was made as follows: 5 kilos. of corn meal, after extraction with water (Preparation H), were treated with 10-per cent. salt solution, and the filtered extract directly dialysed until the salt was removed. The separated proteid was then filtered off, dissolved again in 10-per cent. salt solution, the solution filtered, and once more dialysed until the proteid had completely separated. The globulin so obtained (Preparation H^2), after being washed with alcohol and ether, weighed 5 grams, and on analysis (dried at 110° C.) gave the following results:

Analysis of Corn Globulin, Preparation H².

I. 0.2844 gram substance gave 0.1728 gram H_2O = 6.75 per cent. H, and 0.5196 gram CO_2 = 49.82 per cent. C.

II. 0.4564 gram substance gave 0.2699 gram H_2O = 6.57 per cent. H, and 0.8283 gram CO_2 = 49.49 per cent. C.

III. 0.3892 gram substance gave 57.4 cc. N at 16.3° C. and 759.8 mm. pressure = 17.47 per cent. N.

IV. 0.3767 gram substance gave 56.1 cc. N at 17.4° C. and 755.0 mm. pressure = 17.47 per cent. N.

V. 0.5075 gram substance gave 0.0156 gram ash = 3.07 per cent.

VI. 0.5323 gram substance gave 0.0162 gram ash = 3.04 per cent.

VII. 0.5050 gram substance fused with $KOH + KNO_3$ gave 0.0338 gram $BaSO_4$ = 0.92 per cent. S.

VIII. 0.5315 gram substance fused with $KOH + KNO_3$ gave 0.0345 gram $BaSO_4$ = 0.89 per cent. S.

Percentage Composition of the Ash-free Substance.

							Average.
C	51.38	51.04	..	..	..	..	51.21
H	6.96	6.78	..	..	..	..	6.87
N	..	..	18.02	18.02	..	..	18.02
S	..	..	..	..	0.94	0.91	0.93
O	..	..	..	..	..	..	22.97
							100.00

It is evident from these results that by this process of extraction, first with water and lastly with salt solution, a good separation of the two globulins present in the corn-kernel may be obtained; the myosin-like body passing mainly into the first or aqueous extract, the vitellin dissolving in the 10-per cent. salt solution. When we recall how difficult it many times is to separate closely related proteid bodies, the above method seems fairly successful. In the body last described we have the same globulin (vitellin) which was previously separated from the mixed globulin B, both by heat-coagulation and by what we may term recrystallisation from dilute salt solutions. In their content of nitrogen all of these preparations of vitellin show a close agreement, and, furthermore, a fairly close agreement in carbon. Moreover, the average composition of these products agrees very closely with the generally accepted composition of phyto-vitellin.

The following table shows this relationship very clearly:

	Prep. A[2].	Prep. B[4].	Prep. B[6].	Prep. F[2].	Prep. G[2].	Prep. H[2].	Phyto-vitellin.*
C	51.76	51.94	52.26	51.99	51.10	51.21	51.88
H	6.80	6.81	6.88	6.81	6.90	6.87	7.51
N	18.16	18.22	18.17	18.02	18.13	18.02	18.08
S	23.28	23.06	22.69	0.66	0.98	0.93	0.60
O				22.52	22.89	22.97	21.93

* Prepared from pumpkin seeds and analysed by Barbieri by Weyl's method (J. prakt. Chem. **18**, 102–116).

As is seen from the table of results, the vitellin is noticeably different from the corn myosin in having a smaller percentage of sulphur, the latter containing on an average 1.27 per cent. of

sulphur, while the vitellin contains only about 0.85 per cent. It is also noticeable that preparations G^2 and H^2 contain a lower percentage of carbon than the other products. What the explanation of this is we cannot say, although it is to be remembered that these two preparations of vitellin (unlike F^2) were separated from the salt solution by direct dialysis, without previous precipitation with ammonium sulphate, and possibly are not quite so pure.

As to the respective quantities of these two globulins present in the kernel, it would appear that the vitellin is greatly in excess, although it is to be remembered that the myosin is more readily changed into an insoluble modification by the process of separation, and hence considerable loss occurs.

From the foregoing it is plain that the mixed globulin first described, obtained by direct extraction of corn meal with 10-per cent. salt solution, as preparations A–D, is mainly a mixture of two globulins resembling in composition myosin and vitellin, the composition of the mixed product being readily explainable, in the main, on that hypothesis. The coagulation-points of these two globulins, however, are far from according with the coagulation-points of phyto-myosin and vitellin as usually given. What we have chosen to call corn myosin shows a tendency to coagulate at several points, as already described, but is mainly coagulable at about 70°, while the vitellin-like body in a neutral salt solution appears almost non-coagulable by heat. In fact, the latter body partakes in many respects of the character of hetero-albumose, and is perhaps as closely related to this body as to the true globulins. The myosin-like body is evidently much like the peculiar globulin (not analysed) described by Martin[1] as present in papaw juice, the coagulation-temperatures and general reactions of the two bodies being apparently much the same.

There is every indication of gradual changes being produced by the application of heat to the solutions of these globulins, and, in our opinion, the variable coagulation-points observed are an index of this fact. Sidney Martin[2] has shown that the myosin present in the kernels of wheat and rye is transformed into an insoluble body by simple warming of the 10-per cent. sodium-chloride solution of the globulin at 35°–40° C. for some hours, and Vines[3] claims that phyto-vitellin may undergo transformation into myosin. The peculiar heat-coagulation points of the two

[1] Journal of Physiology **6**, 353. [2] Ibid. **8**, "On two classes of vegetable globulins." [3] Ibid. **3**, 93–114.

corn globulins may possibly be due in part to rapid conversion into the insoluble or albuminate stage and its separation as a coagulum.

There is, however, evidence of still another globulin in the corn kernel, differing from all of the preceding preparations, but, doubtless, sometimes present in small quantity in the products already described.

f. *A more soluble globulin which separates from its solution only after long-continued dialysis.*

The two most important characteristics of a globulin are its solubility in various strengths of salt solution and its insolubility in water; hence the removal of the salt from a globulin containing solution, by dialysis, constitutes one of the most effectual methods of separating such proteids from their solutions, provided the removal of the salts is complete. Under ordinary circumstances a globulin dissolved in a salt solution separates quite rapidly when the solution is subjected to dialysis. Obviously, the rate of separation will depend greatly upon the endosmotic equivalent of the salts present and the solubility of the globulin, but under most circumstances the separation is practically complete some time before every trace of the salt is removed. Sodium chloride diffuses much more rapidly than ammonium sulphate; indeed, the latter salt requires a very long time for its complete removal, even when dialysed in running water of a fair degree of purity. The same is true of the phosphates of the alkalies present in the corn kernel, so that an extract of corn meal, for example, made with 10-per cent. salt solution, may be dialysed until every trace of chloride is removed and still give a reaction for phosphates.

In the separation of the mixed globulins from the corn kernel as already described, it will be remembered that the usual procedure was to precipitate the filtered salt extract of the meal with ammonium sulphate added to saturation. The precipitated proteids were collected on a filter, allowed to drain, and then dissolved so far as possible in water and 10-per cent. solution of sodium chloride. Obviously, such a solution after filtration when ready for dialysis, would contain, in addition to the proteids, anywhere from 5 to 8 per cent. of sodium chloride, and possibly 1 per cent. of ammonium sulphate. In separating the dissolved globulin by dialysis it was our custom to continue the dialysis until the solution failed to give any reaction for chlorides with silver nitrate and nitric acid, considering that when that point was reached the globulin would be completely precipitated. Thus, in the prepa-

ration of globulin B from 25 kilos. of corn meal, after the method just indicated, the dialysis of the sodium-chloride and ammonium-sulphate solution was continued (in running water) for two weeks, at the end of which time the chloride was entirely removed, and over 42 grams of globulin had separated. The clear filtered fluid still contained a trace of sulphate, and as an experiment it was again exposed to dialysis in small parchment bags suspended in running water, for one week longer. Greatly to our surprise, a second deposit of globulin formed, which, after being washed with water, alcohol and ether, weighed, air-dry, 2.8 grams (Preparation B^6).

The filtrate from this product was again returned to the dialysers for ten days longer, when a third deposit of globulin formed, which, after being washed and dried, weighed 1.3 grams (Preparation B^7). The filtrate from this product gave no further deposit even when dialysed in distilled water.

These two preparations were unquestionably true globulins; both were wholly insoluble in water, but dissolved readily in dilute salt solutions. Preparation B^6, dissolved in 10-per cent. solution of sodium chloride, coagulated as follows: the solution grew slightly turbid at 59° and flocked at 62°. The filtrate from this coagulum grew slightly turbid again at 69°, but there was no separation of flocks, even when the mixture was boiled, or on the addition of acetic acid. Evidently, therefore, the substance was precipitated at 62°. Addition of potassium ferrocyanide to the acid fluid, however, gave a slight precipitate, indicating that the substance was not wholly coagulable by heat, or else that some little proteose-like substance had been formed in the process.

The composition of the globulin, after drying at 110° C. until of constant weight, was as follows:

Analysis of Corn Globulin, Preparation B^6.

I. 0.3094 gram substance gave 0.1915 gram H_2O = 6.87 per cent. H, and 0.5911 gram CO_2 = 52.10 per cent. C.

II. 0.3516 gram substance gave 0.2121 gram H_2O = 6.70 per cent. H, and 0.6721 gram CO_2 = 52.13 per cent. C.

III. 0.3895 gram substance gave 47.5 cc. N at 2.5° C. and 756.0 mm. pressure = 15.12 per cent. N.

IV. 0.3798 gram substance gave 46.9 cc. N at 5° C. and 759.4 mm. pressure = 15.22 per cent. N.

V. 0.4427 gram substance fused with $KOH + KNO_3$ gave 0.0407 gram $BaSO_4$ = 1.26 per cent. S.

VI. 0.3895 gram substance gave 0.0019 gram ash = 0.50 per cent.

Percentage Composition of the Ash-free Substance.

						Average.
C	52.36	52.39	..	..	..	52.38
H	6.90	6.74	..	..	..	6.82
N	..	..	15.20	15.30	..	15.25
S	..	..	..	..	1.26	1.26
O	..	..	..	..	..	24.29
						100.00

Judging from the coagulation-point and composition of this product, it is a globulin distinctly different from the preceding preparations. The above data taken together clearly prove that it is not a mere trace of myosin, vitellin, or both, which had escaped separation, although a trace of these bodies may have been mixed with it, as is indeed suggested by the turbidity at 69°, but that it represents a more soluble globulin than either of the preceding, and one containing a much lower percentage of nitrogen. It resembles myosin rather than vitellin, yet differs from them both in composition, in the temperature at which it coagulates, and in its marked solubility in very dilute solutions of salts other than chlorides.

The low content of nitrogen in this globulin is substantiated by the percentage of nitrogen in the second product (B^1), which is undoubtedly the same as the preceding.

Analysis of Corn Globulin, Preparation B^1.

I. 0.3051 gram substance gave 36.6 cc. N at 3.0° C. and 757.2 mm. pressure = 14.84 per cent. N.

II. 0.3987 gram substance gave 0.0085 gram ash = 2.13 per cent.

Percentage of nitrogen in the ash-free substance = 15.16.

Without doubt, traces of this globulin may be carried down with the other globulins, especially with the myosin and with the mixed globulin, and this may be the cause of the low coagulation-point occasionally observed in the other products. On the other hand, if the preliminary dialysis for the separation of the two more insoluble globulins is not continued sufficiently long, then the product obtained on subsequent dialysis will contain some myosin, and possibly vitellin.

This is well illustrated in the following experiment: The proteids precipitated from a sodium-chloride extract of 5 kilos. of corn meal (Preparation E) by ammonium sulphate, and then dissolved

in water and 10-per cent. salt solution, were dialysed in running water for one week, when a considerable separation of globulin occurred. This was filtered off, and the solution, which contained an appreciable amount of salt, was again dialysed for one week longer, yielding 1.16 grams of air-dry globulin (Preparation E^6). The filtrate, which contained a trace of sulphate, was again dialysed for one week, when there resulted a small separation of globulin, weighing, air-dry, 0.25 gram (Preparation E^7). These were dried at 110° C. and analysed with the following results:

Analysis of the Globulin E^6.

I. 0.3728 gram substance gave 48.1 cc. N at 3.0° C. and 768.3 mm. pressure = 16.20 per cent. N.

II. 0.4817 gram substance gave 0.0075 gram ash = 1.56 per cent.

Analysis of the Globulin E^7.

I. 0.1700 gram substance gave 20.6 cc. N at 3.0° C. and 767.7 mm. pressure = 15.21 per cent. N.

Not enough substance for an ash determination.

From this it is sufficiently evident that the first product (E^6) is, as might be expected, a mixture of corn myosin with the more soluble globulin; while the latter product (E^7) corresponds closely, at least in its content of nitrogen, with the preceding products of like nature.

A tabular arrangement of the analyses of what we may term pure preparations of this globulin shows a fairly close agreement in composition.

	Prep. B^6.	Prep. B^7.	Prep. E^7.
C	52.38	..	..
H	6.82	..	..
N	15.25	15.16	15.21
S	1.26	..	..
O	24.29	..	..

From the foregoing descriptions this body is to be considered as a third globulin present in small amount in the corn kernel, and characterised especially by a low content of nitrogen, 15.2 per cent., and with a coagulation-point at about 62° C.

g. Insoluble products derived from the foregoing globulins.

As is well known, many proteid bodies undergo change by long contact with water or by contact with strong salt solutions, being converted thereby into insoluble modifications resembling albumi-

nates, soluble only in dilute alkaline fluids, or, it may be, by longer action, into bodies closely resembling coagulated proteids. This is notably true of animal myosin, of freshly precipitated syntonin, and of certain vegetable globulins. We have found this likewise true of one or more of the corn globulins just described. Thus, when a perfectly clear sodium-chloride extract of corn meal is dialysed and the globulin thereby precipitated, a portion of the product is changed in the process, as evidenced by its being no longer soluble in solutions of sodium chloride of any strength. This insoluble portion, however, is dissolved by dilute solutions of sodium carbonate, being precipitated therefrom on neutralisation, apparently as alkali-albuminate. Again, in precipitating the proteids from a sodium-chloride extract of corn meal by saturation of the solution with ammonium sulphate, a portion of the globulin precipitated thereby loses its solubility in dilute salt solutions, being plainly converted into the same insoluble modification. Further, if by chance the ammonium sulphate used in the precipitation of the proteids is acid, the transformation is far more rapid and complete, apparently in proportion to the acidity. Naturally, there may likewise occur, in this case, some formation of insoluble acid compounds.

The details of the formation and separation of some of these insoluble transformation products are as follows:

1. *Insoluble products formed by the action of ammonium sulphate on the mixed globulin directly extracted from corn by 10-per cent. salt solution.*

In the preparation of the mixed globulin B, 25 kilos. of finely ground corn were extracted with an abundance of 10-per cent. salt solution at the temperature of the room, and the resultant solution filtered through filter-paper, yielding a perfectly clear fluid. All of the proteids were then precipitated by saturation of the solution at the ordinary temperature with recrystallised and perfectly neutral ammonium sulphate. This precipitate was then treated with a small volume of water, sufficient to make with the adherent salt a 1- to 3-per cent. solution of ammonium sulphate, and then with 10-per cent. solution of sodium chloride as long as the latter exerted any solvent action. As a result there remained quite an amount of insoluble matter, wholly insoluble in water and salt solutions, but readily dissolved by solutions of sodium carbonate of sufficient strength. It was, therefore, treated with a solution of

1-per cent. sodium carbonate, the fairly clear fluid filtered through paper, and the proteid reprecipitated by neutralisation with dilute hydrochloric acid. After being washed with water until free from salts, then with alcohol and ether, it weighed, air-dry, 4.7 grams (Preparation B^x). In this connection it may be well to recall the fact that in this extraction of corn there were obtained 42 grams of purified mixed globulin and 4.1 grams of the more soluble globulin, thus indicating that approximately one-tenth of the total amount of globulin was converted into this insoluble modification, presumably through the action of the ammonium sulphate. This alteration-product, after being dried at 110° C., gave on analysis the following results:

Analysis of Preparation B^x.

I. 0.4400 gram substance gave 0.2781 gram H_2O = 7.02 per cent. H, and 0.8657 gram CO_2 = 53.66 per cent. C.

II. 0.3960 gram substance gave 0.2504 gram H_2O = 7.02 per cent. H, and 0.7769 gram CO_2 = 53.51 per cent. C.

III. 0.3447 gram substance gave 44.1 cc. N at 2.0° C. and 763.2 mm. pressure = 16.02 per cent. N.

IV. 0.7012 gram substance fused with $KOH + KNO_3$ gave 0.0557 gram $BaSO_4$ = 1.09 per cent. S.

V. 0.5472 gram substance fused with $KOH + KNO_3$ gave 0.0476 gram $BaSO_4$ = 1.19 per cent. S.

VI. 0.5810 gram substance gave 0.0039 gram ash = 0.67 per cent.

Percentage Composition of the Ash-free Substance.

						Average.
C	54.02	53.87	..	..	..	53.95
H	7.05	7.05	..	..	..	7.05
N	..	..	16.13	..	..	16.13
S	..	..	..	1.09	1.19	1.14
O	..	..	..	..	..	21.73
						100.00

Two similar preparations, each from 5 kilos. of corn meal, were obtained in much the same manner as the preceding, except that, through the inadvertent use of slightly acid ammonium sulphate in the precipitation of the proteids, a far larger proportion of the globulin was converted into this insoluble modification and, further, in both cases the insoluble product contained considerable matter which would not dissolve even in 1- to 3-per cent. sodium carbonate. That portion soluble in 1-per cent. sodium carbonate

was filtered off and in each case precipitated by neutralisation with dilute hydrochloric acid.

The products were then washed free from salts with water, with alcohol, and thoroughly extracted with ether (Preparations M^z and N^z).

On analysis the following results were obtained, which show fairly close agreement with the preceding data:

Analysis of Preparation M^z.

I. 0.3874 gram substance gave 0.2403 gram H_2O = 6.89 per cent. H, and 0.7497 gram CO_2 = 52.77 per cent. C.

II. 0.4460 gram substance gave 0.2749 gram H_2O = 6.85 per cent. H, and 0.8595 gram CO_2 = 52.57 per cent. C.

III. 0.4373 gram substance gave 55.5 cc. N at 7.3° C. and 765.6 mm. pressure = 15.64 per cent. N.

IV. 0.8302 gram substance fused with $KOH + KNO_3$ gave 0.0693 gram $BaSO_4$ = 1.14 per cent. S.

V. 0.6978 gram substance fused with $KOH + KNO_3$ gave 0.0577 gram $BaSO_4$ = 1.13 per cent. S.

VI. 0.9290 gram substance gave 0.0176 gram ash = 1.89 per cent.

Percentage Composition of the Ash-free Substance.

						Average.
C	53.79	53.59	..	..	..	53.69
H	7.02	6.98	..	..	..	7.00
N	..	..	15.94	..	..	15.94
S	..	..	..	1.16	1.15	1.16
O	..	..	..	..	..	22.21
						100.00

Analysis of Preparation N^z.

I. 0.3777 gram substance gave 0.2315 gram H_2O = 6.80 per cent. H, and 0.7174 gram CO_2 = 51.79 per cent. C.

II. 0.3437 gram substance gave 0.2101 gram H_2O = 6.79 per cent. H, and 0.6502 gram CO_2 = 51.59 per cent. C.

III. 0.4419 gram substance gave 56.6 cc. N at 7.4° C. and 763.6 mm. pressure = 15.74 per cent. N.

IV. 0.7266 gram substance fused with $KOH + KNO_3$ gave 0.0609 gram $BaSO_4$ = 1.15 per cent. S.

V. 0.8527 gram substance fused with $KOH + KNO_3$ gave 0.0634 gram $BaSO_4$ = 1.02 per cent. S.

VI. 0.7776 gram substance gave 0.0229 gram ash = 2.94 per cent.

Percentage Composition of the Ash-free Substance.

						Average.
C	53.39	53.19	..	..	..	53.29
H	7.00	6.99	..	..	..	7.00
N	..	..	16.23	..	..	16.23
S	..	..	..	1.18	1.05	1.12
O	..	..	..	..	..	22.36
						100.00

From these three analyses it is plainly manifest that these insoluble products formed in the manner indicated are essentially the same. Their composition, moreover, indicates that in the formation of these bodies a decided alteration occurs, if we assume that they are formed from the mixed globulins already described. In fact, none of the globulins thus far identified could yield products with the high content of carbon noted here without undergoing a change of considerable magnitude. Obviously, we do not know how much of this change in composition results from the action of the ammonium sulphate and how much from the action of the dilute sodium carbonate, but inasmuch as we have obtained from a mixed globulin an insoluble body having the same low content of nitrogen, without the use of the sodium carbonate, it seems fair to presume that the change in composition is independent of any action of the dilute alkaline fluid.

2. *Insoluble products formed by the action of ammonium sulphate on the globulin extracted from corn by water, and on the globulin extracted by 10-per cent. solution of salt after previous extraction of the corn with water.*

In the preparation of extract F, 6.5 kilos. of corn meal were treated with cold water—about 14 litres—in two or more portions, and the filtered solutions precipitated with neutral ammonium sulphate added to saturation. This precipitate was then treated with water and 10-per cent. salt solution as long as anything was dissolved.

The residue was then dissolved in 0.5-per cent. solution of sodium carbonate, filtered, and the proteid precipitated by neutralisation. After being washed with water, alcohol and ether, it weighed, air-dry, 1.35 grams (Preparation F^x).

Analysed, it gave the following results:

Analysis of Preparation F^x.

I. 0.2557 gram substance gave 0.1581 gram $H_2O = 6.86$ per cent. H, and 0.4974 gram $CO_2 = 53.05$ per cent. C.

II. 0.3350 gram substance gave 43.9 cc. N at 3° C. and 759.3 mm. pressure = 16.27 per cent. N.

III. 0.3800 gram substance gave 0.0028 gram ash = 0.73 per cent.

Percentage Composition of the Ash-free Substance.

			Average.
C	53.43	..	53.43
H	6.90	..	6.90
N	..	16.39	16.39
O	..	.. }	23.28
S	..	.. }	
			100.00

The residue of corn meal remaining after the above extraction with water, was then extracted with 10-per cent. solution of sodium chloride, the filtered solution precipitated with ammonium sulphate, and the proteids treated exactly as described under the aqueous extract. As a result, 1.45 grams of insoluble proteid were obtained, which on analysis gave the following results:

Analysis of Preparation F^{v}.

I. 0.4967 gram substance gave 0.3086 gram H_2O = 6.90 per cent. H, and 0.9537 gram CO_2 = 52.36 per cent. C.

II. 0.3520 gram substance gave 45.1 cc. N at 3° C. and 749.1 mm. pressure = 15.69 per cent. N.

III. 0.4165 gram substance gave 0.0049 gram ash = 1.17 per cent.

Percentage Composition of the Ash-free Substance.

			Average.
C	52.98	..	52.98
H	6.98	..	6.98
N	..	15.87	15.87
S	..	.. }	24.17
O	..	.. }	
			100.00

3. *Insoluble products formed through the action of water on the globulins extracted from corn meal by water and by 10-per cent. salt solution.*

The sodium chloride and ammonium sulphate solutions of the globulins filtered off from the two preceding preparations, F^{x} and F^{v}, were dialysed in running water until the salts were practically all removed and the globulins separated from their respective solutions.

On attempting to dissolve these products in 10-per cent. solution of sodium chloride, a certain proportion remained insoluble;

in other words, during the long dialysis a portion of each globulin, formerly soluble in the salt solution, had become insoluble. These insoluble products were filtered off, washed thoroughly with 10-per cent. salt solution, dissolved in 1-per cent. sodium carbonate, the solution filtered and neutralised with dilute hydrochloric acid. The precipitated proteids, after being washed with water, alcohol and ether, weighed, air-dry, respectively 1.2 grams (Preparation F^{xa}) and 1.73 grams (Preparation F^{ya}), the former having come from the globulin extracted by water alone, and hence presumably formed from the myosin-like globulin, while the latter product, F^{ya}, on similar grounds must have been formed from the vitellin-like globulin or from some adherent body.

These two products, on analysis, gave the following results:

Analysis of Preparation F^{xa}.

I. 0.2278 gram substance gave 29.8 cc. N at 2.5° C. and 756.9 mm. pressure = 16.24 per cent. N.

II. 0.3678 gram substance gave 0.0029 gram ash = 0.79 per cent.

Percentage of N in the ash-free substance = 16.37.

Analysis of Preparation F^{ya}.

I. 0.2795 gram substance gave 0.1740 gram H_2O = 6.92 per cent. H, and 0.5302 gram CO_2 = 51.74 per cent. C.

II. 0.2844 gram substance gave 38.3 cc. N at 3.0° C. and 760.3 mm. pressure = 16.74 per cent. N.

III. 0.4025 gram substance gave 0.0018 gram ash = 0.45 per cent.

Percentage Composition of the Ash-free Substance.

			Average.
C	51.97	..	51.97
H	6.95	..	6.95
N	..	16.82	16.82
S	..	.. }	24.26
O	..	.. }	
			100.00

Arranging these several results in tabular form, it becomes easy to draw certain general conclusions from the data:

	B^x.	M^x.	N^x.	F^x.	F^y.	F^{xa}.	F^{ya}.
C	53.95	53.69	53.29	53.43	52.98	..	51.97
H	7.05	7.00	7.00	6.90	6.98	..	6.95
N	16.13	15.94	16.23	16.39	15.87	16.37	16.82
S	1.14	1.16	1.12 }	23.28	24.17	..	24.26
O	21.73	22.21	22.36 }				

	Myosin-like globulin.	Very soluble globulin, B^6.	Vitellin-like globulin, F^2.
C	52.72	52.38	51.99
H	7.05	6.82	6.81
N	16.82	15.25	18.02
S	1.32	1.26	0.66
O	22.05	24.29	22.52

With the exception of the product F^{y_a}, all of these insoluble bodies have the same general composition, and are alike characterised by a high content of carbon. Taking into consideration the percentage of nitrogen in these bodies and the nitrogen of the several globulins present in the corn kernel, it is evident that the vitellin-like globulin cannot well be considered as an antecedent of these insoluble products. These latter bodies are unquestionably formed from the myosin-like globulin and the still more soluble globulin with 15.25 per cent. of nitrogen. Doubtless it is this greater tendency of these two globulins to pass into insoluble modifications which facilitates the purification of the vitellin-like globulin. Thus the like preparations, F^x and F^y, coming as they do from the aqueous extract and from the sodium-chloride extract of the corn respectively, indicate a common origin, and it can be explained only on the supposition (certainly plausible enough) that the myosin-like globulin, not being wholly dissolved by the water extract, is dissolved together with the vitellin by the 10-per cent. salt solution and then later changed into the insoluble modification just described. Again, the content of sulphur is of significance in this connection, in that the myosin-like globulin and its still more soluble neighbor are both characterised by a fairly high content of sulphur, while the vitellin-like globulin contains less than 1 per cent. of this element.

In conclusion, then, we are apparently justified in making the statement that through the long-continued action of water and strong solutions of salt, as ammonium sulphate, the two globulins with a low content of nitrogen are gradually changed into insoluble modifications, which are especially characterised by a relatively high content of carbon.

II.—Proteids Soluble both in Water and in Dilute Salt Solutions.

An aqueous extract of corn meal, as well as a sodium chloride extract, contains in addition to the globulins already described more or less proteid matter soluble in water alone and having the general properties of ordinary albumin and proteose. These bodies can be detected, either in the original extract after the complete removal of all globulin, or better in the ammonium-sulphate precipitate of the mixed proteids, after the globulins and their alteration-products have been entirely separated by dialysis and filtration.

Of albumin-like bodies there would appear to be two present, both more or less coagulable by heat, but unlike in chemical composition. Moreover, one is precipitable by the addition of dilute acetic or hydrochloric acid to a 10-per cent. sodium-chloride solution, after the globulins have been entirely removed, while the other remains in solution and can be advantageously separated only by coagulation of the neutralised fluid. From the albumin-free solution, the proteose can be separated by saturation with sodium chloride and addition of a little acetic, or hydrochloric acid.

An aqueous solution containing these water-soluble proteids is precipitated on saturation with ammonium sulphate, and on addition of alcohol, but not by saturation with sodium chloride. Addition of 0.2-per cent. hydrochloric acid or 30-per cent. acetic acid likewise gives no precipitate. Heated gradually, an aqueous solution of these proteids becomes turbid at about 46°, with separation of a flocculent precipitate at 60°. Between 60° and 70° a fairly large coagulum separates. The fluid again becomes turbid about 85°, and on boiling, a slight coagulum appears which gradually increases as the boiling is continued. In fact, it is very difficult to make a complete coagulation. The solution may be evaporated nearly to dryness, the residue taken up in water and filtered, and then on boiling this solution for some time an additional coagulum may appear. Indeed, there is every evidence of some kind of transformation in the process of coagulation, or possibly it may be that we have to deal simply with a body very slowly, or incompletely, coagulable by heat, like some forms of phyt-albumose.

Preparation A.—The proteids precipitated from a sodium-chloride extract of 5 kilos. of corn meal, by means of ammonium sulphate, were dissolved in water and 10-per cent. solution of sodium chloride, and dialysed until every trace of globulin and soluble salts had been removed. To the clear neutral fluid thus obtained, which gave no precipitate whatever on the addition of dilute acid or on saturation with sodium chloride, pure, crystallised salt was added in such quantity that the solution contained 10 per cent. of sodium chloride, after which the clear fluid was rendered slightly acid with 0.2-per cent. hydrochloric acid. The precipitate thus produced was filtered off, treated with water in which, or in the slightly acid fluid which resulted, it was mainly soluble, and the solution carefully neutralised with a few drops of very dilute sodium carbonate. This gave rise to an abundant precipitate which was filtered off, washed thoroughly with water, alcohol and ether, weighing, when air-dry, 2.48 grams (Albumin A^1). This substance, when first precipitated by neutralisation, was very readily soluble in dilute sodium carbonate, but after becoming dry it was wholly insoluble in the dilute alkaline fluid.

This description affords a very good illustration of the readiness with which some of these vegetable proteids undergo change by mere contact with dilute acids or alkalies. Evidently, the addition of a little 0.2-per cent. acid to the salt solution of these proteids gives rise to an acid compound of one of the albumins insoluble in salt solution (10-per cent.), but soluble in water or, rather, in the slightly acid fluid. Withdrawal of the acid, however, by neutralisation with a few drops of very dilute sodium carbonate, yields not the original albumin, but apparently an insoluble acid-albumin; a reaction more characteristic of a globulin than of a genuine albumin.

The 10-per cent. sodium-chloride solution, from which the above albumin was precipitated by the addition of 0.2-per cent. hydrochloric acid, was heated to boiling, when there resulted a coagulum which, after being washed with hot water until free from salt, and then with alcohol and ether, weighed air-dry, 1.03 grams (Albumin A^2).

On boiling the filtrate from this latter coagulum, no further precipitate could be obtained, but saturation of the slightly acid fluid with salt gave a considerable precipitate, readily soluble in water. The substance thus precipitated was therefore dissolved

in water and dialysed until the sodium chloride was entirely removed. The salt-free solution was then evaporated to dryness on a water-bath. As the solution became concentrated, a portion of the proteid separated as a scum or skin on the surface of the fluid, eventually making an insoluble coagulum. On treating the dried residue with water, still more insoluble matter appeared, showing that either all of the coagulable albumins (Albumins A^1 and A^2) had not been removed from the fluid, or else that in the process of treatment changes were going on by which new coagulable matter was being formed. Eventually, however, by evaporating the filtered fluid to dryness several times and taking up the residue in water, there resulted a solution of a proteid body which showed no signs of coagulation, even on long-continued heating, and which yielded a gummy precipitate on the addition of absolute alcohol. This proteose-like body was, however, obtained in very small quantity—only about 0.3 gram—and might be considered as possibly formed by the hydrolytic action of the repeated evaporations, etc.

On the other hand, it might perhaps be claimed with equal plausibility that the slow coagulations witnessed above were the result of a regressive metamorphism of a proteose normally present in the corn-kernel.

The two albumins, A^1 and A^2, were dried at 110° C. and analysed with the following results:

Analysis of Albumin A^1.

I. 0.3828 gram substance gave 0.2355 gram H_2O = 6.84 per cent. H, and 0.7398 gram CO_2 = 52.71 per cent. C.

II. 0.3454 gram substance gave 43.4 cc. N at 3.0° C. and 761.7 mm. pressure = 15.64 per cent. N.

III. 0.3185 gram substance gave 39.4 cc. N at 2° C. and 768.5 mm. pressure = 15.60 per cent. N.

IV. 0.3292 gram substance gave 0.0010 gram ash = 0.30 per cent.

Percentage Composition of the Ash-free Substance.

				Average.
C	52.86	..	..	52.86
H	6.86	..	..	6.86
N	..	15.69	15.65	15.67
S	..	..	.. }	24.61
O	..	..	.. }	
				100.00

Analysis of Albumin A².

I. 0.2355 gram substance gave 0.1430 gram H_2O = 6.76 per cent. H, and 0.4464 gram CO_2 = 51.72 per cent. C.

II. 0.3498 gram substance gave 45.9 cc. N at 3° C. and 758.5 mm. pressure = 16.27 per cent. N.

III. 0.3214 gram substance gave 0.0013 gram ash = 0.40 per cent.

Percentage Composition of the Ash-free Substance.

			Average.
C	51.93	..	51.93
H	6.79	..	6.79
N	..	16.33	16.33
S	..	.. }	24.95
O	..	.. }	
			100.00

Preparation B.

Another series of similar products was made as follows: 6.5 kilos. of finely ground corn were extracted first with water and then with 10-per cent. salt solution. From these two extracts the proteids were precipitated by saturation with ammonium sulphate, the precipitates dissolved separately, so far as possible, in water and 10-per cent. salt solution, and the globulins separated by thorough dialysis. In the two filtrates, wholly free from globulin, the addition of an equal volume of 20-per cent. salt solution and a little 0.2-per cent. hydrochloric acid produced a flocculent precipitate, considerably heavier in the aqueous extract than in the sodium-chloride extract. As the two precipitates, however, were apparently the same, they were united and dissolved, so far as possible, in water. In this case, unlike the preceding experiment, a large proportion of the precipitate did not dissolve in the water added, apparently because the fluid was not sufficiently acid. This insoluble portion, after being washed with water, was therefore dissolved in a little 0.2-per cent. hydrochloric acid and the solution carefully neutralised with dilute sodium carbonate, by which the substance was reprecipitated. After being washed thoroughly with water, alcohol and ether, it weighed, air-dry, 2.67 grams (Albumin B¹).

On analysis, after drying at 110° C., it yielded the following results:

Analysis of Albumin B¹.

I. 0.3274 gram substance gave 0.1992 gram H_2O = 6.75 per cent. H, and 0.6369 gram CO_2 = 53.05 per cent. C.

II. 0.3133 gram substance gave 0.1900 gram H_2O = 6.74 per cent. H, and 0.6081 gram CO_2 = 52.92 per cent. C.

III. 0.4413 gram substance gave 54.8 cc. N at 20° C. and 762.1 mm. pressure = 15.53 per cent. N.

IV. 0.4758 gram substance gave 0.0048 gram ash = 1.01 per cent.

Percentage Composition of the Ash-free Substance.

				Average.
C	53.58	53.48	..	53.53
H	6.82	6.81	..	6.82
N	..	..	15.69	15.69
S	..	..	.. }	23.96
O	..	..	.. }	
				100.00

The two original acid filtrates, from which the above albumin was separated, were in this preparation directly saturated with sodium chloride, and the precipitate of the residual albumin and proteose filtered off and dissolved in water. The resultant solutions were united, neutralised and dialysed until the salts were entirely removed. At the end of the dialysis, a small amount of substance was found adhering to the walls of the dialyser, not, however, sufficient in quantity to do anything with. On evaporation of the filtered fluid to half its volume considerable coagulum formed, which was washed with water, alcohol and ether, weighing, when air-dry, 1.32 grams (Albumin B²). On further evaporation of the mother-liquor containing the proteose an additional coagulum formed, which was added to the preceding.

This preparation, dried at 110° C., gave on analysis the following results:

Analysis of Albumin B².

I. 0.2387 gram substance gave 0.1453 gram H_2O = 6.76 per cent. H, and 0.4547 gram CO_2 = 51.95 per cent. C.

II. 0.3546 gram substance gave 47.6 cc. N at 3° C. and 751.8 mm. pressure = 16.49 per cent. N.

III. 0.3868 gram substance gave 0.0008 gram ash = 0.21 per cent.

Percentage Composition of the Ash-free Substance.

				Average.
C	52.06	..	..	52.06
H	6.77	..	..	6.77
N	..	16.53	..	16.53
S	..	..	..	24.64
O	..	..	..	
				100.00

In the filtrate from which this last body was obtained there still remained some coagulable matter, which was finally completely removed by careful heating on a water-bath. The concentrated and perfectly neutral fluid was then precipitated with absolute alcohol, and the resultant proteose collected and washed with alcohol and ether.

This substance, like the soluble proteoses formed from animal proteids, was especially characterised by extreme solubility in water, non-coagulability by heat, etc. It was precipitated by acetic acid and potassium ferrocyanide, by alcohol, phosphotungstic acid, etc., and gave the usual proteid reactions with Millon's reagent, and with cupric sulphate and potassium hydroxide.

Dried at 110° C. until of constant weight, the product, separated as just described, gave on analysis the following results:

Analysis of Proteose B.

I. 0.3390 gram substance gave 0.1952 gram H_2O = 6.40 per cent. H, and 0.6154 gram CO_2 = 49.51 per cent. C.

II. 0.3409 gram substance gave 0.1993 gram H_2O = 6.49 per cent. H, and 0.6158 gram CO_2 = 49.27 per cent. C.

III. 0.3326 gram substance gave 41.3 cc. N. at 2.5° C. and 760.9 mm. pressure = 15.49 per cent. N.

IV. 0.2984 gram substance gave 37.4 cc. N at 4° C. and 758.5 mm. pressure = 15.49 per cent. N.

V. 0.2784 gram substance, fused with $KOH + KNO_3$, gave 0.0520 gram $BaSO_4$ = 2.57 per cent. S; deducting 0.25 for S in the ash = 2.32 per cent. S.

VI. 0.4206 gram substance gave 0.0120 gram ash = 2.42 per cent.

Percentage Composition of the Ash-free Substance.

						Average.
C	50.74	50.50	..	..	..	50.62
H	6.56	6.65	..	..	..	6.61
N	..	..	15.88	15.88	..	15.88
S	..	..	..	..	2.37	2.37
O	..	..	..	..	..	24.52
						100.00

Preparation C.

These same bodies were prepared again, on a somewhat larger scale, from 25 kilos. of finely ground corn. The process was as follows: The meal was extracted directly with an abundance of 10-per cent. salt solution, and the proteids precipitated collectively by saturation of the fluid with ammonium sulphate. The precipitate was dissolved in water and salt solution, and the fluid dialysed about three weeks, until the salts were entirely removed and the globulins completely separated.

The salt-free solution, containing the albumins and proteose, was then treated with sodium chloride until the mixture contained 10 per cent. of salt, after which 0.2-per cent. hydrochloric acid was added until the precipitation was complete. The albumin so separated was filtered off and treated with water, in which about four-fifths of it dissolved. The residue, however, was dissolved in a little 0.2-per cent. hydrochloric acid and the two solutions united, and dialysed until free from chlorides. At the end of the dialysis the fluid was perfectly neutral, and the albumin (obviously altered), or a portion of it, was found precipitated in the parchment tube, the same as on neutralisation of the slightly acid fluid described in the previous preparations, while in the solution was found considerable non-coagulable proteose which must have been carried down with the albumin. The precipitate (Albumin C[1]) was filtered off, washed thoroughly with water, alcohol and ether, and dried at 110° C. for analysis. Air-dry, it weighed 3.14 grams.

Analysis of Albumin C[1].

I. 0.3459 gram substance gave 0.2102 gram H_2O = 6.75 per cent. H, and 0.6714 gram CO_2 = 52.93 per cent. C.

II. 0.3649 gram substance gave 0.2222 gram H_2O = 6.77 per cent. H, and 0.7065 gram CO_2 = 52.81 per cent. C.

III. 0.3546 gram substance gave 43 cc. N at 774.0 mm. pressure = 15.35 per cent. N.

IV. 0.6957 gram substance fused with $KOH + KNO_3$ gave 0.0750 gram $BaSO_4 = 1.48$ per cent. S.

V. 0.4331 gram substance gave 0.0016 gram ash = 0.37 per cent.

Percentage Composition of the Ash-free Substance.

					Average.
C	53.12	53.00	..	..	53.06
H	6.78	6.80	..	..	6.79
N	..	..	15.41	..	15.41
S	..	..	..	1.48	1.48
O	..	..	..	..	23.26
					100.00

The proteose precipitated with the above albumin was separated from the concentrated solution, after removal of all coagulable matter, by precipitation with absolute alcohol. It weighed, air-dry, 2.5 grams and gave on analysis, after drying at 110° C., the following results:

Analysis of Proteose C[1].

I. 0.3131 gram substance gave 0.1903 gram $H_2O = 6.75$ per cent. H, and 0.5717 gram $CO_2 = 49.80$ per cent. C.

II. 0.3783 gram substance gave 0.2276 gram $H_2O = 6.69$ per cent. H, and 0.6873 gram $CO_2 = 49.55$ per cent. C.

III. 0.3397 gram substance gave 43.5 cc. N at 3° C. and 770.7 mm. pressure = 16.13 per cent. N.

IV. 0.7246 gram substance fused with $KOH + KNO_3$ gave 0.0837 gram $BaSO_4 = 1.58$ per cent. S.

V. 0.4632 gram substance gave 0.0131 gram ash = 2.83 per cent.

Percentage Composition of the Ash-free Substance.

					Average.
C	51.25	51.00	..	..	51.13
H	6.94	6.88	..	..	6.91
N	..	..	16.59	..	16.59
S	..	..	..	1.62	1.62
O	..	..	..	..	23.75
					100.00

The original filtrate from the precipitate produced by dilute hydrochloric acid in the presence of 10-per cent. sodium chloride was dialysed until nearly free from salt, and then, being perfectly neutral to test-papers, was concentrated to one-third its volume and filtered from the coagulum which resulted. This coagulum

(Albumin C^2) was washed thoroughly with water, alcohol and ether, and after being dried at 110° C. was analysed. Air-dry it weighed 2.28 grams.

Analysis of Albumin C^2, First Coagulum.

I. 0.3226 gram substance gave 0.1927 gram H_2O = 6.64 per cent. H, and 0.6092 gram CO_2 = 51.50 per cent. C.

II. 0.3293 gram substance gave 0.1985 gram H_2O = 6.69 per cent. H, and 0.6195 gram CO_2 = 51.31 per cent. C.

III. 0.3737 gram substance gave 46.3 cc. N at 3° C. and 774.2 mm. pressure = 15.68 per cent. N.

IV. 0.4015 gram substance gave 0.0038 gram ash = 0.94 per cent.

Percentage Composition of the Ash-free Substance.

				Average.
C	51.97	51.78	..	51.88
H	6.70	6.75	..	6.73
N	..	..	15.89	15.89
S	..	..	.. }	25.50
O	..	..	.. }	
				100.00

The filtrate from the above coagulum was concentrated still further, without giving any appreciable coagulum, then saturated with salt and precipitated by the addition of 0.2-per cent. hydrochloric acid. The precipitate was dissolved in water, dialysed until the salt had been entirely removed, and then evaporated to dryness on the water-bath. During this last evaporation a considerable coagulum formed, which was collected, washed, dried and analysed. It weighed 1.22 grams (Albumin C^2, second coagulum).

Analysis of Albumin C^2, Second Coagulum.

I. 0.4488 gram substance gave 0.2600 gram H_2O = 6.44 per cent. H, and 0.8224 gram CO_2 = 49.98 per cent. C.

II. 0.3050 gram substance gave 41.8 cc. N at 3° C. and 755.8 mm. pressure = 16.93 per cent. N.

III. 0.3769 gram substance gave 0.0077 gram ash = 2.04 per cent.

Percentage Composition of the Ash-free Substance.

				Average.
C	51.02	..	..	51.02
H	6.57	..	..	6.57
N	..	17.28	..	17.28
S	..	..	.. }	25.13
O	..	..	.. }	
				100.00

From the concentrated filtrate from this second coagulum, a small amount of proteose was separated by addition of absolute alcohol. Dried at 110° and analysed, it gave the following results:

Analysis of Proteose C^2.

I. 0.3666 gram substance gave 0.2065 gram H_2O = 6.26 per cent. H, and 0.6435 gram CO_2 = 47.87 per cent. C.

II. 0.3333 gram substance gave 0.1901 gram H_2O = 6.34 per cent. H, and 0.5934 gram CO_2 = 48.55 per cent. C.

III. 0.3654 gram substance gave 46.3 cc. N at 3° C. and 768.9 mm. pressure = 15.93 per cent. N.

IV. 0.4266 gram substance gave 0.0159 gram ash = 3.72 per cent.

Percentage Composition of the Ash-free Substance.

				Average.
C	49.72	50.42	..	50.07
H	6.50	6.58	..	6.54
N	..	..	16.54	16.54
S	..	..	.. }	26.85
O	..	..	.. }	
				100.00

Preparation D.

Another experiment after the same order as the preceding was carried out without, however, yielding sufficient of any one product for analysis, except of the albumin coagulable by heat present in the filtrate from the precipitate produced by 10-per cent. salt solution and dilute acid.

This product (Albumin D^2) was analysed for the sake of additional confirmatory evidence, with the following results:

Analysis of Albumin D^2, Coagulum.

I. 0.4274 gram substance gave 0.2584 gram H_2O = 6.71 per cent. H, and 0.8055 gram CO_2 = 51.39 per cent. C.

II. 0.3735 gram substance gave 0.2278 gram H_2O = 6.77 per cent. H, and 0.7076 gram CO_2 = 51.66 per cent. C.

III. 0.3739 gram substance gave 49.8 cc. N at 17.3° C. and 757 mm. pressure = 15.67 per cent. N.

IV. 0.3783 gram substance gave 51.1 cc. N at 18.2° C. and 755.1 mm. pressure = 15.80 per cent N.

V. 0.3450 gram substance gave 0.0013 gram ash = 0.38 per cent.

Percentage Composition of the Ash-free Substance.

					Average.
C	51.58	51.84	..	..	51.71
H	6.73	6.79	..	..	6.76
N	..	..	15.72	15.85	15.78
S, O	..	..	..	..	25.75
					100.00

The relationship in composition of these several products can be seen from the following table:

Albumin Precipitated by Salt and Acid.

	A^1.	B^1.	C^1.
C	52.86	53.53	53.06
H	6.86	6.82	6.79
N	15.67	15.69	15.41
S	24.61	23.96	1.48
O			23.26

Albumin Coagulated by Heat.

	A^2.	B^2.	C^2, 1st.	C^2, 2d.	D^2.
C	51.93	52.06	51.88	51.02	51.71
H	6.79	6.77	6.73	6.57	6.76
N	16.33	16.53	15.89	17.28	15.78
S, O	24.95	24.64	25.50	25.13	25.75

Proteose.

	B.	C^1.	C^2.
C	50.62	51.13	50.07
H	6.61	6.91	6.54
N	15.88	16.59	16.54
S	2.37	1.62	26.85
O	24.52	23.75	

We are so firmly convinced that these bodies are, in part at least, the products of changes induced by the several steps incidental to their separation, that we are loth to draw any very definite conclusions as to their true chemical composition. By this we do not mean that these three series of products are wholly artificial. Bodies of the general nature of the albumins here indicated unquestionably exist in the corn-kernel. As already stated, an aqueous solution of the corn proteids, entirely freed from globulins, gives a coagulum between 60° and 70°, and another one between 85° and 100°, thus implying the presence of two distinct bodies. It may

possibly be assumed that the albumin precipitated by salt and acid is simply a portion of the albumin separated later by coagulation; that this partial precipitation is simply another illustration of the incompleteness characteristic of the separation of these albumins, as witnessed, for example, in the gradual and incomplete coagulation of the above albumin by heat; and further, that the difference in chemical composition between the first and second series of products is simply due to their alteration by the processes made use of. Yet we can hardly consider this the true view. The peculiar heat-coagulation phenomena of the original aqueous solution, before any separations were attempted, lend favor to the view that at least two coagulable albumins are present. On the other hand, the incompleteness of the coagulation at any one point—the fact that more and more coagulum continues to form as the boiling is continued—points clearly to gradual changes, which, doubtless, leave their impression upon the products separated. Thus, in Preparation C, the two albumins coagulated by heat, C^2 1st and C^2 2d, should theoretically have the same composition, the only tangible difference being that the second coagulum required longer heating to bring about its separation, and yet it differs from its neighbor by 1.5 per cent. of nitrogen. Observations of this character, and others which we will not take space to detail, confirm us in the opinion that these soluble bodies are exceedingly prone to change, and doubtless the composition here given is only an approximation to the true make-up of the substances as they exist in the corn-kernel. At the same time, it may not be amiss to recall the fact that in the seed itself, in its various stages of growth and development, kindred changes are doubtless taking place among its natural proteid constituents. Whether the proteose separated by the above methods exists as such in the corn-kernel, or whether it is derived from the other proteids by the methods of separation, is likewise uncertain. A portion of it is unquestionably formed by the hydrolytic changes incidental to the separation of the coagulable proteids, and this favors the view that it is wholly an artificial product.

Further, a similar proteose-like body was directly prepared by heating to 80° C. one of the preceding globulins, dissolved in salt-solution. Again, the somewhat variable composition of the proteoses analysed suggests a mixture of two or more dissimilar proteoses, such as might be expected in an artificial production of the character indicated.

One point to be noted in connection with the proteose is its relatively low content of carbon; a point which is characteristic of most of the albumoses or proteoses formed by the action of the digestive ferments, both from animal and vegetable proteids.

III.—Proteid Matter Soluble in Alcohol, but Insoluble in Water and Salt-Solutions.

Under this head we have to deal with only one substance, viz. *Zein* or the "maize fibrin" of Ritthausen. The former name, originally given by Gorham[1] to this peculiar proteid found by him in *Zea mays*, seems especially appropriate, and we would suggest its adoption as a more fitting name than the term employed by Ritthausen. This body, as is well known, is especially characterised by its solubility in weak alcohol, and can be obtained by direct extraction of corn meal with 95-per cent. alcohol, preferably at 40°–60°C., or by extraction of the meal with warm alcohol after previous treatment with water, salt-solution, or both.

Several preparations were made under varying conditions as follows:

Preparation A.

2.5 kilos. of finely ground corn meal were first thoroughly extracted with cold water, after which the residue of meal was treated with 95-per cent. alcohol in sufficient quantity to have the water retained by the meal reduce the strength to about 75 per cent. The mixture was warmed for some hours at about 46° C., and finally filtered at approximately that temperature. The filtrate was concentrated on the water-bath at a temperature below 65° C. The residue of meal was again treated with 75-per cent. alcohol (4 litres) at 60°C. for several hours, and filtered while at that temperature. In all, five successive extractions were made after this fashion, the individual alcoholic extracts being evaporated separately, all at a low temperature. The last three extracts left but little residue on evaporation. As the first two extracts became concentrated the proteid separated as a tough, leathery, yellow-colored mass on the sides and bottom of the dish. It was cut up into small pieces and soaked for several days in cold absolute alcohol. On this first treatment of the proteid with absolute alcohol some little dissolved, presumably from dilution of the

[1] See Berzelius, Jahresbericht 2, (1822), p. 124.

alcohol with the water contained in the substance. This alcoholic extract was therefore concentrated to a syrup and the proteid precipitated by ether. It then had the appearance of a crumbly, pulverisable substance, and was easily ground to a powder under absolute alcohol. On slightly warming the alcohol, however, the substance melted and finally dissolved completely in the alcoholic fluid. On cooling this solution, the substance separated in a very gummy state.

The main portion of the proteid, which had been treated once with absolute alcohol, was treated with successive portions of cold absolute alcohol, as long as anything was dissolved by this reagent, after which it was treated with a mixture of one part of ether and two parts of absolute alcohol and, finally, was soaked in ether alone. After this treatment we were able to reduce it to a fine powder, when it was thoroughly extracted with ether for the complete removal of any adhering fat.

So prepared, the substance was only partially soluble in warm 75-per cent. alcohol, a portion of it during the foregoing process having been converted into an insoluble modification. This change from the soluble (in warm alcohol) to the insoluble form is liable to occur whenever the substance is heated with too watery alcohol, especially if the temperature approaches 100° C., or if the heating be long-continued. The soluble portion referred to above was therefore completely separated by repeated treatment with warm and with boiling dilute alcohol, and the insoluble portion ultimately washed with absolute alcohol and ether and dried at 110° C. for analysis.

The alcoholic solution of the soluble portion of the proteid was poured into about three volumes of water, by which the substance was precipitated in white flocks, which, however, soon commenced to unite and rise to the surface, eventually forming a dense cake. On heating this mass it continued to contract, squeezing out the water in large quantities. The substance, still containing considerable water, was next dissolved in warm 95-per cent. alcohol, the solution filtered, concentrated to about 400 cc. and again poured into a large volume of water, about 3 litres. This time the substance did not separate as before, but remained suspended in the form of a thick emulsion and was made to flock only on the addition of a little salt. It was then filtered off, washed with

water until free from chlorides, lastly with alcohol and ether and dried at 110° C. for analysis.

Analysis of Zein. Preparation A.—Soluble.

I. 0.3079 gram substance gave 0.2045 gram H_2O = 7.34 per cent. H, and 0.6226 gram CO_2 = 55.14 per cent. C.

II. 0.3453 gram substance gave 46.8 cc. N at 17.0° C. and 757.9 mm. pressure = 15.98 per cent. N.

III. 0.5310 gram substance fused with KOH + KNO_3 gave 0.0197 gram $BaSO_4$ = 0.51 per cent. S.

IV. 1.0210 grams substance fused with OH + KNO_3 gave 0.0402 gram $BaSO_4$ = 0.54 per cent. S.

V. 1.0600 grams substance gave 0.0024 gram ash = 0.22 per cent.

VI. 1.0045 grams substance gave 0.0028 gram ash = 0.27 per cent.

Percentage Composition of the Ash-free Substance.

					Average.
C	55.27	..	..	..	55.27
H	7.35	..	..	..	7.35
N	..	16.01	..	..	16.01
S	..	..	0.51	0.54	0.53
O	..	..	..	..	20.84
					100.00

Analysis of Zein. Preparation A.—Insoluble.

I. 0.3210 gram substance gave 0.2094 gram H_2O = 7.24 per cent. H, and 0.6451 gram CO_2 = 54.80 per cent. C.

II. 0.4055 gram substance gave 0.2680 gram H_2O = 7.34 per cent. H, and 0.8170 gram CO_2 = 54.94 per cent. C.

III. 0.3171 gram substance gave 43.1 cc. N at 17.1° C. and 761.6 mm. pressure = 16.10 per cent. N.

IV. 0.3250 gram substance gave 43.7 cc. N at 16.8° C. and 763.5 mm. pressure = 15.98 per cent. N.

V. 0.5000 gram substance fused with KOH + KNO_3 gave 0.0221 gram $BaSO_4$ = 0.61 per cent. S.

VI. 0.9834 gram substance gave 0.0018 gram ash = 0.18 per cent.

VII. 0.2637 gram substance gave 0.0005 gram ash = 0.19 per cent.

Percentage Composition of the Ash-free Substance.

						Average.
C	54.90	55.04	..	..	..	54.97
H	7.25	7.35	..	..	..	7.30
N	..	..	16.01	16.13	..	16.07
S	..	..	..	..	0.61	0.61
O	..	..	..	..	..	21.05
						100.00

Preparation B.

2.5 kilos. of finely ground corn, which had been thoroughly extracted with 10-per cent. salt solution, were treated directly with warm 75-per cent. alcohol, until the alcohol ceased to dissolve anything further. The first alcoholic extract, on concentration, yielded the proteid—doubtless on account of the large amount of sodium chloride it contained—as a yellow, granular mass which could be broken quite readily. The residues from the following extracts, however, were of the same general nature as those described under Preparation A. All of the residues from the several extracts were united, washed with distilled water until the salt was entirely removed, then dissolved in warm 80-per cent. alcohol and the solution filtered to remove any insoluble matter present. The solution was next evaporated on a water-bath nearly to dryness and the residue pressed as dry as possible, yielding a yellow, elastic substance which could be pulled like molasses candy, but which was much more elastic and tough. Its elasticity was so great that it could be pulled into sheets several inches square and very thin. After continued kneading under absolute alcohol, it finally became brittle, a considerable portion of it passing into solution at the same time. After thorough extraction with absolute alcohol in this manner, it was soaked for some time in ether and then ground to a coarse powder. Like the preceding preparation, the proteid was now found only partially soluble in dilute alcohol. It was therefore separated into two portions by continued treatment with warm 80-per cent. alcohol, after first moistening the powdered product with a little water. The insoluble portion was washed with absolute alcohol and ether, and dried at 110° C. for analysis.

The alcoholic solution containing the soluble portion of the proteid was poured into three volumes of water, by which the zein was precipitated in white flocks, which gradually formed into a cake floating on the surface of the fluid. This was redissolved in 95-per cent. alcohol, and again precipitated as before. After a third precipitation in this manner, the substance was dissolved in 95-per cent. alcohol, the solution filtered, and the filtrate evaporated on the water-bath. Towards the end, absolute alcohol was added several times in order to prevent the substance from separating in consequence of the alcohol becoming too dilute. The

evaporation was finally carried to dryness, and the residue cut up into small pieces and thoroughly extracted with ether. A portion of this soluble zein, dried at 110° C. until of constant weight, was analysed with the following results:

Analysis of Zein. Preparation B.—Soluble.

I. 0.3252 gram substance gave 0.2132 gram H_2O = 7.28 per cent. H, and 0.6550 gram CO_2 = 54.92 per cent. C.

II. 0.2968 gram substance gave 0.1932 gram H_2O = 7.24 per cent. H, and 0.5985 gram CO_2 = 54.98 per cent. C.

III. 0.4193 gram substance gave 57.4 cc. N at 17.3° C. and 755.1 mm. pressure = 16.06 per cent. N.

IV. 0.3569 gram substance gave 49.5 cc. N at 18.4° C. and 755.1 mm. pressure = 16.20 per cent. N.

V. 0.5365 gram substance gave 0.0025 gram ash = 0.46 per cent.

VI. 0.4590 gram substance gave 0.0020 gram ash = 0.46 per cent.

VII. 1.0270 grams substance fused with $KOH + KNO_3$ gave 0.0423 gram $BaSO_4$ = 0.57 per cent. S.

VIII. 1.0093 grams substance fused with $KOH + KNO_3$ gave 0.0385 gram $BaSO_4$ = 0.52 per cent. S.

Percentage Composition of the Ash-free Substance.

							Average.
C	55.17	55.23	..	..	..	..	55.20
H	7.31	7.27	..	..	..	..	7.29
N	..	..	16.13	16.27	..	..	16.20
S	..	..	..	..	0.57	0.52	0.55
O	..	..	..	..	..	..	20.76
							100.00

The insoluble portion, after being dried at 110° C., gave the following results on analysis:

Analysis of Zein. Preparation B.—Insoluble.

I. 0.3562 gram substance gave 0.2290 gram H_2O = 7.14 per cent. H, and 0.7200 gram CO_2 = 55.12 per cent. C.

II. 0.4284 gram substance gave 0.2772 gram H_2O = 7.19 per cent. H, and 0.8660 gram CO_2 = 55.12 per cent. C.

III. 0.4203 gram substance gave 0.2708 gram H_2O = 7.15 per cent. H, and 0.8504 gram CO_2 = 55.17 per cent. C.

IV. 0.3733 gram substance gave 51.2 cc. N at 17.5° C. and 762.4 mm. pressure = 16.24 per cent. N.

V. 0.4449 gram substance gave 60.8 cc. N at 16.9° C. and 761.8 mm. pressure = 16.20 per cent. N.

VI. 0.5238 gram substance fused with $KOH + KNO_3$ gave 0.0245 gram $BaSO_4$ = 0.64 per cent. S.

VII. 0.7747 gram substance fused with $KOH + KNO_3$ gave 0.0350 gram $BaSO_4 = 0.62$ per cent. S.

VIII. 0.4675 gram substance gave 0.0021 gram ash = 0.45 per cent.

IX. 0.4705 gram substance gave 0.0020 gram ash = 0.43 per cent.

Percentage Composition of the Ash-free Substance.

								Average.
C	55.36	55.36	55.41	..	..	..	..	55.37
H	7.17	7.22	7.18	..	..	..	..	7.19
N	..	..	..	16.31	16.27	..	..	16.29
S	..	..	..	..	..	0.64	0.62	0.63
O	..	..	..	..	..	..	..	20.52
								100.00

Preparation C.

2.5 kilos. of corn meal, previously extracted with 5-per cent. ammonium chloride solution, were treated several times with warm 75-per cent. alcohol, and the alcoholic extract concentrated at a low temperature. The leathery-like residues left by evaporation of the alcohol were washed somewhat with water, then dissolved, so far as possible, in warm 80-per cent. alcohol, the clear fluid evaporated nearly to dryness, and the residue digested repeatedly with absolute alcohol and, lastly, with ether. On becoming air-dry, the substance was pulverised and again extracted with ether. It was then dissolved as completely as possible in 80-per cent. alcohol, after having been first gently warmed with a little water. A considerable quantity of the substance remaining insoluble, this was removed by decantation and filtration, the residue washed first with dilute alcohol, lastly with alcohol and ether, and dried at 110° C. for analysis. The alcoholic solution containing the soluble portion of the proteid was precipitated by being poured into water, a process which was repeated three times; after which the precipitated zein was digested with absolute alcohol and finally with ether, and a portion dried at 110° C. for analysis.

Analysis of Zein. Preparation C.—Soluble.

I. 0.3728 gram substance gave 0.2396 gram $H_2O = 7.14$ per cent. H, and 0.7520 gram $CO_2 = 55.01$ per cent. C.

II. 0.3587 gram substance gave 0.2305 gram $H_2O = 7.14$ per cent. H, and 0.7236 gram $CO_2 = 55.01$ per cent. C.

III. 0.4218 gram substance gave 58.0 cc. N at 17.2° C. and 759.6 mm. pressure = 16.24 per cent. N.

IV. 0.4085 gram substance gave 55.9 cc. N at 17.2° C. and 760.2 mm. pressure = 16.17 per cent. N.

V. 1.0375 grams substance fused with $KOH + KNO_3$ gave 0.0460 gram $BaSO_4 = 0.61$ per cent. S.

VI. 0.8267 gram substance fused with $KOH + KNO_3$ gave 0.0348 gram $BaSO_4 = 0.58$ per cent. S.

VII. 0.4610 gram substance gave 0.0008 gram ash $= 0.17$ per cent.

VIII. 0.5250 gram substance gave 0.0009 gram ash $= 0.17$ per cent.

Percentage Composition of the Ash-free Substance.

							Average.
C	55.10	55.10	..	..	..	..	55.10
H	7.15	7.15	..	..	..	..	7.15
N	..	..	16.26	16.19	..	..	16.22
S	..	..	..	..	0.61	0.58	0.60
O	..	..	..	..	..	..	20.93
							100.00

Analysis of Zein, Preparation C.—Insoluble.

I. 0.3885 gram substance gave 0.2518 gram $H_2O = 7.20$ per cent. H, and 0.7835 gram $CO_2 = 54.99$ per cent. C.

II. 0.3848 gram substance gave 0.2514 gram $H_2O = 7.25$ per cent. H, and 54.94 per cent. C.

III. 0.4964 gram substance gave 67.9 cc. N at 17.5° C. and 763.6 mm. pressure = 16.22 per cent. N.

IV. 0.4119 gram substance gave 57.0 cc. N at 17.7° C. and 760.6 mm. pressure = 16.33 per cent. N.

V. 0.5295 gram substance fused with $KOH + KNO_3$ gave 0.0240 gram $BaSO_4 = 0.62$ per cent. S.

VI. 0.5890 gram substance fused with KOH KNO_3 gave 0.0273 gram $BaSO_4 = 0.64$ per cent. S.

VII. 0.5250 gram substance gave 0.0014 gram ash $= 0.27$ per cent.

VIII. 0.5737 gram substance gave 0.0013 gram ash $= 0.23$ per cent.

Percentage Composition of the Ash-free Substance.

							Average.
C	55.12	55.07	..	..	..	..	55.10
H	7.21	7.26	..	..	..	..	7.23
N	..	..	16.26	16.37	..	..	16.31
S	..	..	..	..	0.62	0.64	0.63
O	..	..	..	..	..	..	20.93
							100.00

Preparation D.

3.0 kilos. of freshly ground corn were extracted directly with about 4 litres of 75-per cent. alcohol for 24 hours, at a temperature varying between 40° and 60° C., and filtered while warm. The clear extract was then concentrated to about 500 cc. and the

proteid precipitated by pouring the solution into a large volume of water. The substance so separated, in the form of lumps and strings, was washed frequently with a large amount of water, and finally soaked in water for some hours. It was then collected on muslin, squeezed dry, treated with 95-per cent. alcohol, in which it dissolved readily and almost completely, and again precipitated by water. This process of solution in dilute alcohol and reprecipitation with water was repeated several times, the product ultimately being divided into two fractions, which differed from each other simply in the number of times this process had been repeated. The two products, D^1 and D^2, were then treated repeatedly with absolute alcohol and ether, until all coloring matter and fat had been removed, after which they were ground to a fine powder and portions dried at 110° C. for analysis.

The residue of meal, extracted a second time with warm dilute alcohol, yielded a second portion of zein, which was separated and purified in essentially the same manner as the preceding preparation, the final product being wholly soluble in dilute alcohol. This was likewise reduced to a powder, after successive treatment with ether and absolute alcohol, and a portion dried at 110° C. for analysis. (Product D^3.)

Analysis of Zein. Preparation D^1.

I. 0.3028 gram substance gave 0.1991 gram H_2O = 7.30 per cent. H, and 0.6107 gram CO_2 = 54.99 per cent. C.

II. 0.2455 gram substance gave 0.1590 gram H_2O = 7.20 per cent. H, and 0.4952 gram CO_2 = 55.00 per cent. C.

III. 0.3580 gram substance gave 47.8 cc. N at 16.8° C. and 766.2 mm. pressure = 15.92 per cent. N.

IV. 0.3557 gram substance gave 48.3 cc. N at 18.4° C. and 756.3 mm. pressure = 15.90 per cent. N.

V. 0.5000 gram substance fused with $KOH + KNO_3$ gave 0.0234 gram $BaSO_4$ = 0.64 per cent. S.

VI. 0.5145 gram substance gave 0.0040 gram ash = 0.78 per cent.

Percentage Composition of the Ash-free Substance.

						Average.
C	55.42	55.43	..	..	..	55.42
H	7.35	7.25	..	..	..	7.30
N	..	..	16.04	16.02	..	16.03
S	..	..	..	..	0.64	0.64
O	..	..	..	..	..	20.61
						100.00

Analysis of Zein. Preparation D².

I. 0.3401 gram substance gave 0.2220 gram H_2O = 7.25 per cent. H, and 0.6857 gram CO_2 = 54.98 per cent. C.

II. 0.3333 gram substance gave 0.2156 gram H_2O = 7.19 per cent. H, and 0.6740 gram CO_2 = 55.14 per cent. C.

III. 0.3696 gram substance gave 50.1 cc. N at 17.0° C. and 757.4 mm. pressure = 15.97 per cent. N.

IV. 0.3731 gram substance gave 50.5 cc. N at 17.9° C. and 755.7 mm. pressure = 15.89 per cent. N.

V. 0.6198 gram substance fused with KOH + KNO_3 gave 0.0305 gram $BaSO_4$ = 0.67 per cent. S.

VI. 0.5065 gram substance gave 0.0030 gram ash = 0.59 per cent.

VII. 0.5131 gram substance gave 0.0035 gram ash = 0.68 per cent.

Percentage Composition of the Ash-free Substance.

						Average.
C	55.33	55.49	..	..	..	55.41
H	7.29	7.23	..	..	..	7.26
N	..	..	16.07	15.99	..	16.03
S	..	..	..	..	0.67	0.67
O	..	..	..	..	..	20.63
						100.00

Analysis of Zein. Preparation D³.

I. 0.3638 gram substance gave 0.2366 gram H_2O = 7.22 per cent. H, and 0.7300 gram CO_2 = 54.72 per cent. C.

II. 0.3892 gram substance gave 0.2551 gram H_2O = 7.27 per cent. H, and 0.7846 gram CO_2 = 54.99 per cent. C.

III. 0.4819 gram substance gave 65.3 cc. N at 17.9° C. and 761.2 mm. pressure = 15.99 per cent. N.

IV. 0.4143 gram substance gave 55.6 cc. N at 17.0° C. and 759.7 mm. pressure = 15.86 per cent. N.

V. 0.5200 gram substance gave 0.0038 gram ash = 0.73 per cent.

VI. 0.5163 gram substance gave 0.0039 gram ash = 0.75 per cent.

VII. 1.0117 grams substance fused with KOH + KNO_3 gave 0.0395 gram $BaSO_4$ = 0.54 per cent. S.

VIII. 1.0520 grams substance fused with KOH + KNO_3 gave 0.0425 gram $BaSO_4$ = 0.56 per cent. S.

Percentage Composition of the Ash-free Substance.

							Average.
C	55.12	55.38	..	..	..	..	55.25
H	7.27	7.32	..	..	..	..	7.29
N	..	..	16.10	15.97	..	..	16.04
S	..	..	..	..	0.54	0.56	0.55
O	..	..	..	..	..	..	20.87
							100.00

A comparison of the analyses of these several preparations of zein, or maize fibrin, shows a remarkable uniformity in composition, and furthermore indicates that the insoluble modification of the proteid is not at all different in composition from the main product. This is plainly apparent from the following table:

	Soluble Zein.						*Insoluble Modification.*			
	A	B	C	D^1	D^2	D^3	A	B	C	Average.
C	55.27	55.20	55.10	55.42	55.41	55.25	54.97	55.37	55.10	55.23
H	7.35	7.29	7.15	7.30	7.26	7.29	7.30	7.19	7.23	7.26
N	16.01	16.20	16.22	16.03	16.03	16.04	16.07	16.29	16.31	16.13
S	0.53	0.55	0.60	0.64	0.67	0.55	0.61	0.63	0.63	0.60
O	20.84	20.76	20.93	20.61	20.63	20 87	21.05	20.52	20.73	20.78
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Ash	0.24	0.46	0.17	0.78	0.63	0.74	0.19	0.44	0.25	

Maize fibrin, as prepared and analysed by Ritthausen,[1] was found to contain 54.69 per cent. C, 7.51 per cent. H, 16.33 per cent. N and 0.69 per cent. S, thus agreeing very closely with our data, except in carbon. This slight difference in composition obviously cannot be explained with certainty, but considering the great care used in the purification of our products and their close agreement in composition, together with the use of unquestionable analytical methods, it seems probable that the above series of data must accurately represent the true composition of maize fibrin or zein. It is furthermore equally evident from the analytical data that this substance is a homogeneous body, and not composed of two or more dissimilar bodies.

Freshly precipitated zein (precipitated from an alcoholic solution by water) is wholly insoluble in 0.5-per cent. sodium carbonate, even when warmed with the alkaline fluid at 40° C. for 24 hours. It is likewise equally insoluble in 0.2-per cent. hydrochloric acid, but can be completely dissolved by an aqueous solution of potassium hydroxide of 0.2-per cent. strength. So dissolved it is not converted into alkali-albumin, as is indicated by the fact that the precipitate produced on neutralisation of the alkaline fluid is not soluble in excess of dilute acid, but is completely dissolved by alcohol. This resistance of the proteid to the

[1] "Ueber den Stickstoffgehalt der Pflanzen-Eiweisskörper."—Pflüger's Archiv für die gesammte Physiologie **18**, 246.

action of alkalies is quite pronounced; thus, exposure of the substance to the action of 0.2-per cent. caustic potash at 40° C. for 24 hours is not followed by any conversion into alkali-albumin. This is likewise true even when the strength of the alkali is raised to 2.0-per cent. KOH. Exposure to a higher temperature than 40° C. with this strength of alkali is, however, followed by a change in the proteid.

Freshly precipitated zein is wholly insoluble in water, and on being boiled with water is changed into the insoluble modification already referred to, which is insoluble both in alcohol and dilute (0.2-per cent.) potassium hydroxide. Zein gives the usual proteid reactions with Millon's xanthoprotein and biuret tests.

On warming the proteid at 40° C. with pepsin-hydrochloric acid, the substance is gradually dissolved and converted into proteose-like bodies, and true peptones non-precipitable by saturation with ammonium sulphate.

Boiled with dilute sulphuric acid (6 cc. concentrated acid in 300 cc. water) zein at first melts, forming a gummy mass or lump which is only slowly attacked by the acid, the portion dissolved being mainly converted into proteoses and peptone. Heated with stronger sulphuric acid, it undergoes a more pronounced decomposition, yielding large amounts of leucin with considerable tyrosin, and apparently large amounts of glutamic acid.

Corn meal, after thorough extraction with salt-solution and warm dilute alcohol, yields little proteid matter to dilute solutions of potassium hydroxide (0.2-per cent.).

Summary.

1. The maize-kernel contains several distinct proteids, well characterised in reactions and composition. Of these, there are three globulins, one or more albumins, and an alcohol-soluble proteid.

2. The globulin obtainable from the maize-kernel by extraction with 10-per cent. solution of sodium chloride and separation by the usual methods—as by dialysis, or by precipitation with ammonium sulphate, followed by dialysis—is a mixture of two or more dissimilar globulins, differing from each other both in composition and in coagulation-points.

3. The mixed globulin can be approximately separated into its

two constituents by fractional heat-coagulation, or by a process of "recrystallisation" from warm dilute salt-solution. In the former process, there is at the same time formed a small amount of proteose-like bodies, presumably by hydrolysis of the less resistant globulin.

4. The two globulins separable by the above methods from the mixed globulin are a myosin-like body and a vitellin-like body.

The myosin-like globulin is characterised by containing about 16.8 per cent. of nitrogen and 1.2 per cent. of sulphur, agreeing closely in its composition with animal myosin. It has, however, a coagulation-point (in 10-per cent. salt solution) of about 70° C.

The vitellin-like globulin, on the other hand, contains about 18.1 per cent. of nitrogen and 0.85 per cent. of sulphur, agreeing closely with the generally accepted composition of phyto-vitellin. This body, however, is almost entirely non-coagulable by heat when dissolved in dilute salt-solution, except in the presence of acetic acid. It is more soluble in warm salt-solutions than in cold, and when separated from the former by cooling the fluid, or on dialysis, almost invariably appears in the form of small spheroids.

5. These two globulins exist as such in the corn or maize kernel, and are not products of a cleavage of the so-called mixed globulin. This is evident from the coagulation-points of a salt-solution of the mixed globulin; from the fact that the separation can be accomplished without the aid of heat; and, lastly, since it is possible to directly extract the individual globulins from the kernel, by the use of appropriate solvents.

6. Direct extraction of finely powdered corn meal with water yields a dilute salt-solution, which dissolves the myosin-like globulin, leaving the bulk of the vitellin-like substance undissolved. Probably, the character of the salts present in the kernel plays an important part in this separation. From this solution the myosin can be separated in a fair degree of purity by the usual methods.

7. Extraction of corn meal with 10-per cent. salt solution, after previous extraction with water, dissolves the vitellin-like globulin, from which solution it can be separated by the customary methods. So prepared, it agrees exactly with the vitellin separated by heat-coagulation from the mixed globulin.

8. The third globulin present in the maize-kernel is characterised by extreme solubility in very dilute salt-solutions, especially phosphates and sulphates. It separates from such solutions only

by prolonged dialysis—*i. e.* not until nearly every trace of the above salts has been removed. It coagulates (in a 10-per cent. salt solution) in the neighborhood of 62° C., and contains 15.2 per cent. of nitrogen and 1.26 per cent. of sulphur.

9. Through the long-continued action of water, and also of strong solutions of salt, as ammonium sulphate, the myosin-like globulin, and the globulin with a still lower content of nitrogen, are changed into insoluble modifications, soluble, however, in 0.5-per cent. sodium carbonate solution, from which they are precipitated on neutralisation, apparently as albuminates. So prepared, these insoluble modifications are characterised by a relatively high content of carbon.

10. An aqueous extract of corn meal, as well as a sodium-chloride extract, contains, in addition to the globulins, apparently two albumin-like bodies, more or less coagulable by heat, but as prepared, unlike in chemical composition.

11. A certain amount of proteose can be detected in the extracts of corn meal, after the globulins and albumins have been entirely removed, but apparently this is mainly, if not wholly, an artificial product resulting from the hydrolysis of some one or more of the preceding bodies.

12. Especially noteworthy is the presence in the maize-kernel of a peculiar proteid body known as maize fibrin, or better as *zein*, soluble in warm dilute alcohol, but insoluble in water.

Zein is characterised by a high content of carbon, by its resistance to the action of dilute alkalies (*i. e.*, non-convertibility into alkali-albuminate), and by the ease with which it is converted into an insoluble modification on being warmed with water, or with very weak alcohol.

Soluble zein and the insoluble modification have the same chemical composition. Both respond to the ordinary proteid reactions.